

How can polluters be made to pay?

The doctrine that polluters and other environmental desperadoes should be required to pay for the damage they do is beguiling, but first requires general agreement on broad philosophical principles unlikely soon to be reached.

DR David Pearce, professor of economics at University College, London, scored a big success last week when the new British Secretary of State for the Environment, Mr Christopher Patten, cautiously approved a report which, among other things, argues that the rational protection of the environment requires a system by which environmental assets and natural resources are valued financially, so that their use (or the damage done to them) can be taxed on an equitable basis. The proposition appears to be equitable both between people now alive and those likely to be alive at future epochs. But it is unworkable except in the most restricted fields.

That polluters should pay is a sound principle, nominally the basis of British environmental policy for decades, but more often honoured in the breach than the observance. Although Patten's predecessors have paid lip-service to it, they have usually found that there is no sound basis on which charges can be levied. In principle, the managers of a water-course might argue that those discharging effluents should pay charges proportional to the biological oxygen demand (BOD) of their materials. But what is to be done about inorganic pollutants, for example? And should latecomers to a river-bank pay more than early arrivals because of the probable non-linear effects in the absorptive capacity of the water-course? In Britain, in practice, the application of even this simple principle is too often thwarted by the fact that the managers of watercourses (the statutory water boards) are also the chief sources of BOD (as sewage).

The problems are more serious when comparing different kinds of pollution, for example the discharges to the atmosphere from nuclear power stations and those burning fossil fuel. Nuclear power stations unavoidably generate quantities of radioactive isotopes of the rare gases, mostly argon, which contribute to radioactivity in the atmosphere and which as a consequence may increase the incidence of lung cancer, but to a degree that is hardly large enough to be called marginal compared with natural causes of radiation (leaving cigarette-smoking aside). But fossil-fuel power generation is much more hazardous: apart from being one cause of acid rain, it is also a substantial cause of ill-health, perhaps even premature death, from bronchial diseases. But what politician, these days, is likely to take up the cause of taxing nuclear generating plants less heavily than more old-fashioned plants, however compelling the logic?

The balance between one generation and its successors is even harder to strike sensibly. Take, for example, the present use of petroleum, the reserves of which are finite (in the sense that the quantities that could be produced at present prices must be limited). So it is possible to argue that the present generation should tax petroleum so as to discourage its use, leaving some part of the present stock for the next generation. But that would turn out to be a self-defeating self-denial if, in the next century or so, some other substantial source of energy makes its appearance. And, quite apart from thermonuclear fusion, are there not solar power, wave energy and windmill energy at the front of many people's minds?

The essential weakness of the Pearce proposition is that, while it may make sense in restricted fields, it quickly becomes a nonsense when there is no objective basis for evaluating the damage done by pollution or other environmental insults. There can be no substantial basis, for example, for putting a price on the continued survival of whales or tigers, but such a price would probably be negative (whales eat krill, which might otherwise support commercial fisheries, while tigers kill people among other creatures). And there is a further weakness underlying this beguiling but illfounded argument: since the ultimate cause of environmental damage is people, should births be taxed? And on what basis? □

Poland's way ahead

The new regime in Poland has to walk a delicate tight-rope. Here is how well-wishers can help.

THE irony that the appointment of Poland's first Solidarity prime minister (but Mr Tadeusz Mazowiecki has not yet formed a government) should almost coincide with the fiftieth anniversary of the treaty between the Soviet Union and the Third Reich might almost have been deliberate. That treaty is now acknowledged to have included a hitherto secret protocol in which the two signatories agreed on the partition of Central Europe, and of Poland in particular. A shrunken Poland has been part of the Eastern bloc, and a member of the Warsaw Pact, ever since. Well-wishers should keep in mind that it will remain so for a very long time, perhaps indefinitely.

That, of course, is why Polish politicians have been



treading so delicately in the past few dramatic weeks. Solidarity, which began life as a trade union representing shipyard workers in Gdansk, and which has been illegal for much of its time, has shown all the signs of not wishing to form Poland's next government. It has been forced into accepting what many regard as a poisoned chalice (but it is not) by the failure of the Polish Communist Party to do so, which is in turn a consequence of the unwillingness of the Polish parliament (called the Sejm) to accept the arrangements proposed. That circumstance will itself surprise many in the West with an over-simple view of how the governments of Eastern Europe are organized. Although the Sejm may have been newly legitimized by last June's semi-free elections, it is at least a parliament that, on this occasion, has shown independence.

Luckily, everybody in Poland seems fully aware of the difficulties the developments of the past week have created for the other members of the Warsaw Pact, the Soviet Union included. In the real world, Mazowiecki cannot risk signs of wishing to leave the Eastern bloc without bringing down not only himself but also Mr Mikhail Gorbachev. The practical problem that he faces is whether he can win enough freedom to deal with Poland's pressing economic problems within a framework in which his motives will be deeply suspect. A firm assurance of indefinite devotion to Comecon, Eastern Europe's less democratic version of the European Community, is a necessary precondition of survival.

What, in the circumstances, can people elsewhere do? The conventional view is that there is little room for manoeuvre. But that is what governments say. Offers of large amounts of credit to help with Poland's economic difficulties could easily become, in a few months or years, unpaid loans to a Polish government of quite a different complexion. Even so, there is a case for much more generosity than has been offered so far, if only to sustain hope during the harsh winter that lies ahead. But this is also an unprecedented time, when an apparently sovereign state appears openly to acknowledge that it is at a loss to know how to get out of the mess in which it finds itself. These may be circumstances in which the intellectual community in the West is more able to respond imaginatively than Western governments. A decade ago, the Brandt Commission made some constructive suggestions about the place of developing countries in the modern world, and had an important influence on public policy. Has the time come for the equivalent of a Brandt Commission for Eastern Europe? □

Funny money

British ambitions to find a better convertible currency than the European Commission's await fulfilment.

WESTERN Europe needs to invent some kind of currency that can be used in all 12 member countries of the European Community by the end of 1992, but has not yet

agreed how that should be done.

More precisely, the European Commission last year proposed that the European Currency Unit (ECU) should become a common currency, and that there should be a Central Bank to manage its affairs, but the British government objected at June's summit meeting in Lisbon, since when West Germany has been having second thoughts. But the British government's promise to devise an alternative scheme has not yet lived up to realistic expectations, at least to judge from the British Treasury's whispered information at the weekend.

That there are serious objections to the Commission's proposals cannot be denied. Much would depend on the degree of independence enjoyed by the proposed Central Bank. If, for example, it had a large measure of autonomy, as does the Federal Reserve Bank in the United States or the Bundesbank in West Germany, there would be endless complaints from member governments that the bank was following over-conservative (or over-open) policies. If, on the other hand, the bank were politically controlled, there would be endless squabbling among the members about the correct policy to be followed. European hardliners say, with some justice, that such difficulties are inseparable from the goal of economic unity.

The British government's alternative has the merit of being imaginative, even intriguing. The principle is that existing national currencies should be allowed to compete with each other in the market places in which goods of various kinds are traded for banknotes of different denominations, but that all European currencies should simultaneously be legal tender everywhere. The argument is that national prestige would persuade governments to manage their currencies in such a way that they were considered sound, and that the soundest currency would eventually prevail.

But that is an over-sanguine view. While sellers of goods would have an interest in being paid in the currency most likely to keep its value from one day to the next, purchasers would have exactly the opposite interest. A kind of Gresham's Law would apply.

Quite apart from the horrendous difficulties arising under the British government's proposals when people from different parts of Europe sought to buy cups of coffee and other trivial goods in their national currencies, there are bound to be serious objections to any scheme that tempts either party to a commercial bargain to argue about the currency as well as the price. And would banks as well as traders be required to convert one currency into another without charge, which would be equitable in the circumstances? But there are merits of flexibility in the British proposals. The best compromise would be simultaneously to make the ECU and individual national currencies legal tender everywhere, thus allowing those who buy and sell things in Western Europe to deliver a vote of confidence in their own governments' conduct of economic affairs whenever they go into a shop. In the last resort, that would also be a constraint on the conduct of the otherwise inevitable European Central Bank. □

AIDS closer to becoming a treatable disease

- Antibody-CD4 chimera in clinical trials
- AZT effective in most cases of HIV infection

Washington

AUGUST has so far been a good month for news of AIDS therapies. Three weeks ago, AZT was shown to delay the progression of AIDS in those with AIDS-related complex, and last week it was shown to postpone the development of symptoms in those whose T-cell counts had dropped below 500. This week, clinical trials are set to begin of another emerging treatment that could be used with AZT to lengthen the lives of those infected with HIV (human immunodeficiency virus). Together with AZT, the hybrid antibody-CD4 molecule termed 'immunoadhesin' offers hope that HIV infection may in future be treated as a chronic disease that can be managed for years before it ultimately become fatal.

Immunoadhesin is a genetically engineered molecule composed of part of the CD4 receptor — the entry point through which HIV infects cells — and the back end of the IgG1 antibody, responsible for activating killer cells in the immune system and initiating a virus-attacking system within the blood named complement. Genentech, the company developing immunoadhesin, has shown that it blocks the infection of CD4-bearing cells in culture, and stays in the blood of rabbits for more than four days (see *Nature* 337, 525; 1989).

Soluble forms of the CD4 receptor alone — being tested by Genentech and Biogen — stay in the bloodstream for only roughly an hour when injected directly into the veins of humans. Partly because it is larger, immunoadhesin is expected to last 20–50 times longer, requiring fewer injections for patients. Unlike plain soluble CD4, immunoadhesin may also kill infected cells and traverse the placental barrier, allowing it to be used to protect the fetuses of HIV-infected pregnant women.

The clinical trial of immunoadhesin will be conducted by Genentech's collaborators at the US National Cancer Institute (NCI), the New England Deaconess Hospital in Boston, Massachusetts, San Francisco General Hospital, the University of Washington and Stanford University. The drug will be tested for safety in a total of 30–40 patients given a range of dosages intravenously. The first patient will be treated this week by Samuel Broder's group at NCI — just six months after the initial publication of immunoadhesin, a speed Broder says is "astounding". If the results are encouraging, efficacy studies could begin within a year.

Genentech is now planning controlled efficacy trials for its first-generation soluble CD4. Since the federal approval of AZT two years ago, all drugs being tested in people with AIDS have been compared to AZT, limiting placebo-controlled trials to those with a less advanced stage of HIV disease. But the announcements that AZT is effective in people with AIDS-related complex and in some asymptomatic HIV-infected individuals will mean that AZT will now be the standard of comparison for nearly all trials of drugs to treat HIV disease.

The manufacturer of AZT, Burroughs-Wellcome, is expected to use data from the new AZT studies to ask the US Food and Drug Administration to broaden the

HIPPARCOS

ESA tries to salvage Hipparcos

Munich

AFTER nearly two weeks of unsuccessful attempts to fire the apogee motor on the European astronomy satellite Hipparcos, project leaders have reluctantly begun to design a salvage operation for the mission. The 1,140-kg satellite is currently in an elliptical orbit between 200 km and 35,000 km from the Earth. Project scientist M.A.C. Perryman says the chances are "very small" that Hipparcos can be brought into a geostationary orbit by the motor, as was originally intended (see *Nature* 340, 491; 1989).

One or two more attempts to fire the motor will be made on 24 or 25 August. If they fail, small thrusters on the satellite will be fired early next week to bring the satellite into a higher orbit at a minimum distance of about 600 km from Earth. The thrusters were originally meant to be used to correct for nutation (wobbling) once the satellite was in geostationary orbit.

Hipparcos was designed to measure the precise positions, parallaxes and proper motions of stars. It was expected over two and a half years to measure the positions of 120,000 stars with a precision of 0.002 arcseconds. A second mission on board, called Tycho, was meant to measure the positions of 400,000 stars with an accuracy of 0.03 arcseconds by 1995.

In the current orbit, Hipparcos could last from six months to two years, depending on how fast the solar cells that power the satellite degrade. In six months, the satellite could achieve only one-third the accuracy initially projected, although this

conditions the company can specify in the drug's labelling. Anticipating that AZT will now be used to treat a much wider spectrum of those with HIV disease, stockholders bid up Wellcome plc's stock price by 32 per cent by the end of last week.

But the drug still costs \$8,000–\$10,000 for a year's treatment at a full dose. Jude Payne of the Health Insurance Association of America says "almost all" US health insurers will begin paying for AZT for those with AIDS-related complex and HIV-infected people with T-cell counts of less than 500, based on the new studies. Senator Ted Kennedy (Democrat, Massachusetts) plans to add an amendment to next year's appropriations bill for the US Department of Health and Human Services which would provide \$30 million for three years to pay for AZT for people with AIDS who have no insurance. When Congress returns from its summer recess, there will be pressure for Kennedy's amendment to be extended to cover all those with HIV infection.

Carol Ezzell

would still improve on ground-based results by a factor of 15.

ESA has not decided to ask its 13 member states for the money to build and launch a second Hipparcos satellite identical to the first. But a preliminary decision is expected "in one month", said Roger Bonnet, director of the ESA scientific programmes, after the extent of the degradation of the solar cells has been determined. The ESA council would have to approach the scientific programme committee to request more money. A replacement satellite would cost an estimated £114 million (\$182 million).

It would be difficult to reapportion the money out of the existing ESA science programme budget, which would require a two-thirds majority vote in the committee. But it would be even more difficult to gain a new appropriation for a second Hipparcos, which would require unanimous support. The length of time in orbit is crucial. In six months, only 8 or 10 observations of each star would be possible instead of the 80 originally planned. If Hipparcos were to survive longer, say for a year or a year and a half, proper motions of stars and parallaxes could begin to be measured. But Perryman said that was not a "realistic" expectation.

In its elliptical orbit, the satellite passes every ten hours through the Van Allen belts, where it is subject to irradiation by protons and electrons in the solar wind 5,000 km above the equator. This radiation degrades the solar panels which power the satellite.

Steven Dickman

Judge backs technique

Washington

IN the New York double-murder trial that has become a test case for the forensic use of DNA 'genetic fingerprinting', Justice Gerald Sheindlin last week ruled that the identification techniques are generally reliable and admissible as evidence. Genetic fingerprinting thus emerges from what Sheindlin described as its "most comprehensive and extensive legal examination . . . in the United States" with backing for its use both to demonstrate exclusions (that two samples are not the same) and inclusions (the more difficult test that two samples are identical).

The decision comes with the recommendations that, in future trials, "a pre-trial hearing should be conducted to determine if the testing laboratory substantially performed the scientifically accepted tests and techniques, yielding sufficiently reliable tests to be admissible". In the particular case under examination, Sheindlin ruled that the testing laboratory had, on one inclusion test, "failed in several major respects to use the generally accepted scientific techniques and experiments for obtaining reliable results".

Peter Neufeld, one of the defence lawyers in the case, believes that despite the new recommendations, the judge "did not go far enough" in his criticisms. When the judge decided the evidence was in a general sense reliable, Neufeld says, he was "responding to the hopefulness of our expert witnesses rather than their assessment that these standards and procedures were currently in place". But Neufeld adds that, as the case represents "the very first time that any judge has excluded DNA evidence of a match, on the grounds that the tests were not done in a sufficiently reliable fashion, it has tremendous impact on testing laboratories nationwide".

The DNA identification evidence is of vital significance in the trial of Joseph Castro, a 38-year-old resident of the Bronx, accused of stabbing to death a 20-year-old pregnant woman and her two-year-old daughter. A wrist-watch worn by Castro at the time of his arrest appeared to carry a bloodstain. Castro claimed that the blood was his own. DNA identification tests were performed by the forensic and paternity testing company, Lifecodes Corporation of Valhalla, New York, and were claimed to show that the blood came from the murdered woman rather than Castro.

A pre-trial hearing examined whether the scientific procedures involved met the 'Fyre' test of being "generally accepted as reliable" by the scientific community. The hearing quickly turned into an intense examination of forensic DNA tests in general and of the methods used by Lifecodes Corporation in this particular case.

Approximately 5,000 pages of evidence were amassed over the 12 weeks and a series of distinguished scientists testified for the prosecution and for the defence. The hearing took an unusual twist when defence and prosecution witnesses got together and agreed that some of the evidence was not scientifically reliable.

Although there was speculation that the judge might rule DNA testing inadmissible, the final decision accepts its general validity. In the particular case under consideration, the ruling accepted that Lifecodes Corporation showed that the blood on the watch did not come from the defendant. But the judge ruled inadmissible the company's evidence that the blood matched that of the victim.

Despite the implied criticism of their procedures, Lifecode's senior vice-president John Winkler said he was "delighted that the judge recognized that using DNA for forensic testing is valid". Winkler also accepted the judge's view that "a preliminary, critical examination of the actual testing procedures performed in a particular case" should be conducted before the start of a trial employing DNA identification tests. The judge notes that the test results are bound to have a "powerful impact" on a jury and makes twelve recommendations of what should be presented at a pre-trial hearing.

Winkler says that Lifecodes "agrees with all of the judge's recommendations". Among them are details of the method used to declare a match or nonmatch between samples, the methods used to calculate the allele frequency in the relevant population and tests performed to determine the origin of contaminants. Evidence on all these points was strongly debated during the Castro hearing (see *Nature* 339, 501; 15 June 1989).

Comprehensive guidelines for the forensic use of DNA fingerprinting are still lacking, and the National Academy of Sciences is still hoping to find funds for a detailed study. Meanwhile the Federal Bureau of Investigation (FBI), whose laboratories see themselves as leaders in the forensic DNA analysis field, has brought together molecular biologists, testing-laboratory representatives and its own experts to exchange viewpoints.

Neufeld says, however, that state and federal legislation is necessary. Included should be accreditation of laboratories, certification and licensing of personnel, quality control standards, and proficiency studies.

"It's absurd", says Neufeld "to think that you do not have all that in a testing procedure which determines who lives and who dies, or at a minimum who goes away to prison for 30 years".

Alun Anderson

Japanese scientists stay clear of China for time being

Kyoto & Tokyo

JAPAN'S Ministry of Education, Science and Culture (MESC) has been quietly cutting off the flow of Japanese scientists to China in the wake of the Tiananmen Square killings. Although the ministry has made no official announcement, information on travel grant applications released earlier this month show that visits to China are no longer winning support.

Immediately after the imposition of martial law in China, Japanese government officials and academics involved in academic exchange with China said only that they were "watching the situation" and had no immediate plans to suspend their exchange programmes.

But, it has now emerged that on 6 June, MESC sent a directive to all national universities and research institutes advising against visits to China. The directive is not legally binding but has had the effect of bringing visits by Japanese academics to China to a virtual halt.

A group of mineralogists at Kyoto University led by Professor Shohei Banno had been expecting to receive a grant for a long-planned collaborative project with the Chinese Academy of Sciences to look for unusual minerals in eastern China. But the grant was not among those announced this month. Private funds are available for the trip but because permission for leave of absence must be obtained from the university, the trip cannot go ahead.

Figures provided last week by the Japan Society for the Promotion of Science (JSPS), a semi-governmental organization funded by MESC, also show that the flow of scientists to China has fallen to a trickle. Normally about 30 to 50 Japanese scientists visit China each year under JSPS programmes. But since 4 June only one scientist has been despatched and many have postponed their trips, according to a JSPS spokesman.

The ministry is particularly anxious to avoid having Japanese scientists in Beijing, where martial law is still in force, according to Rinjin Ogasawara, a MESC spokesman on China. Grants that have already been awarded for trips to Beijing can be used for journeys elsewhere, including other countries, or they can be returned to the ministry, as some have been, Ogasawara says.

In the future, however, it seems likely that the flow of Japanese scientists to China (excluding Beijing) will resume. The foreign ministry announced last week that it is withdrawing its advice against Japanese nationals travelling to China, except Beijing, and economic assistance will also be resumed.

Simon Wallis & David Swinbanks

REMOTE SENSING

French back Spot satellite

Paris

FRANCE has decided to back its commercial remote-sensing programme until the end of the century by giving the go-ahead to build a fourth Spot satellite. The decision, announced after a cabinet meeting last week, should mean an extra FF200 million (\$31 million) in next year's budget for the national space research centre (CNES).

At a time when the US Landsat remote-sensing satellites have been given only a temporary reprieve pending a review by the new Bush administration (see *Nature* 338, 365; 1989), the French government is more than ever committed to Spot.

New political interest in environmental issues, together with France's long-standing enthusiasm for space technology, makes the Spot programme an ideal vehicle for national prestige. But Spot-1, launched in 1986, has already overrun its intended life by one year and CNES has decided to take up its option to launch Spot-2 in November aboard an Ariane 40 rocket. CNES had originally hoped to delay the launch so long as Spot-1 continued to function, in order to save money.

Spot-4 embodies some significant new features compared with its predecessors, Spot-1, 2 and 3, which will make it more useful for monitoring changes in vegetation throughout the world. Spot-1's two high-resolution visible (HRV) charge-coupled sensors will be updated and their bandwidth extended to the medium infrared. The new sensors will be made by the French company Thomson instead of the US Fairchild which supplied Spot-1, 2 and 3. The switch to a French company, say the satellite's makers, Matra-Espace, should allow better control of component quality. Interestingly, Matra last week acquired three of Fairchild's communications and space divisions. Spot-4's two data tape-recorders will also be made in France, by Enertec, instead of by the US company Odetics. One of the two Odetics recorders aboard Spot-1 has failed, leaving the satellite unable to send pictures of some areas of the globe.

The change to French manufacturers could also be because a military satellite, Helios, due to be launched in 1993, shares its chassis and many components with Spot-4. The technology will also be useful in the development of the European polar platform contribution to the Freedom space platform. These factors may have helped secure government support.

Despite the 'green' aspect of the decision to back Spot-4, it will be less useful to monitor environmental change than its original design intended. A multispectrum 'vegetation' instrument with a scanning width of 2,220 km and a resolution of 60 km, which would have made possible

global change monitoring, has been dropped. Additional data-processing facilities, both within the satellite and on the ground, would have increased costs by as much as FF1,000 million.

Construction costs for Spot-4 are down to about FF3,000 million, compared with

the FF5,000 million for Spot-1, and its life has been extended from two to four years. Nevertheless, Paul Quilès, minister for posts, telecommunications and space, has said that sales of Spot pictures should balance operating costs (FF210 million in 1988) by 1993.

Spot-4 is scheduled for a 1995 launch, but should be ready to take over if Spot-3, due for launch in 1992, fails. **Peter Coles**

SEISMOLOGY

US-Soviet earthquake collaboration

San Francisco

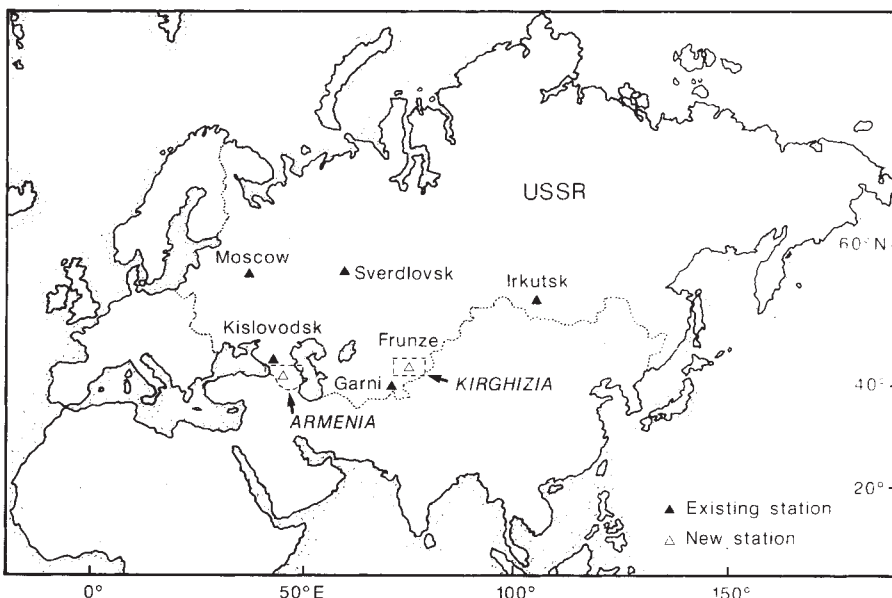
A COMPREHENSIVE scientific agreement between the United States and Soviet Union will make possible two new monitoring stations inside the Soviet Union to help fill in what has long been a 'seismic hole' in a worldwide earthquake-detection network.

The US-Eurasian Seismic Studies Program also allows the upgrading of five existing Soviet seismic listening stations. Altogether, the new efforts are expected to improve earthquake prediction and provide a much more detailed picture of how seismic waves propagate through the Earth in the region (see map). The data will also provide useful background in attempts to monitor underground nuclear tests.

The new agreement marks a joint effort between the US Geological Survey, the

crude circle, comprise part of a 23-station global network called the International Deployment of Accelerometers that is managed by Scripps for IRIS. The new stations will fill in holes in that network by providing the first comprehensive look at two active earthquake zones. One will be installed near Frunze in the south central Soviet Union, where tectonic plates meet to form the boundary of the Indian and Asian subcontinents. The other will be in Garni, just west of the Caspian Sea near the site of the devastating earthquakes that rocked Soviet Armenia last December.

Neither station will be within 1,000 kilometres of a Soviet nuclear test ground, and will therefore have no direct bearing on the monitoring of nuclear tests, according to Scripps geophysicist Bernard Minster. But



Soviet Academy of Sciences and the Incorporated Research Institutions for Seismology (IRIS), a consortium of more than 60 US universities. The five-year programme, supported by the Defense Advanced Research Project Agency (DARPA), includes a first-year budget of \$5.7 million. Most of that money will go to the Scripps Institution of Oceanography, part of the University of California at San Diego, which will coordinate and manage all IRIS operations in the Soviet Union.

The five existing sites, scattered in a

he stresses that the improved model of the Earth, and of how seismic waves travel through it, will indirectly bear on any verification process. "By learning more about the Earth we all get information that will be usable by people who worry about nuclear monitoring", Minster says.

As part of the agreement, Soviet researchers will receive data from five already existing seismic stations in the United States and two new sites to be established in New Mexico and Virginia.

Robert Buder

Germans debate maglev train Go-ahead for

Munich

THE future of West Germany's plans to build a magnetically levitated train called 'Transrapid' is looking increasingly uncertain. Transport Minister Friedrich Zimmermann said recently that his ministry would not spend "a single deutsche mark" on Transrapid. Zimmermann fears that the system would compete with the state-run conventional railway system, from which the government recently assumed DM12,600 million of debt.

The Research Ministry has already invested DM1,200 million (about \$615 million) in the project, but it has now withdrawn its support. A further DM3,000 million in long-term loans is needed to finance the next phase.

Initial plans were for a route connecting Hamburg and Hanover, reducing the train travel time for the 155-kilometre distance from 80 minutes to just 30–40 minutes. But a second stretch in the Ruhr area linking Essen and Bonn is now being considered. The West German cabinet is expected to debate the matter in October.

The consortium to build Transrapid is led by Thyssen Henschel and includes Messerschmitt-Bölkow-Blohm and several of the largest banks in West Germany. The consortium has itself invested about DM100 million in the project so far.

Thyssen admits that Transrapid can be profitable in West Germany only if two distant cities are connected. It has recently raised the stakes by offering to build a 1,025-km track between Hamburg in the north and Munich in the south, looping

past the Ruhr and Rhine valleys on the way. Such a track could cost between DM30,000 million and DM45,000 million. It would take at least 15 years to complete, not counting the time spent fighting court battles against environmentalist opponents of the project.

Although in the 1960s maglev trains were thought of as the best way to link major European cities, the technology has been turned down by the French, the British and others in favour of improving conventional railways. Even West Germany has invested heavily in improving its high-speed intercity rail network with trains that can travel up to 250 kilometres an hour.

Thyssen conducted a publicity campaign this summer to try to win support for Transrapid. Spokesman Lutz Dreesbach said that Transrapid is meant to complement the conventional rail network, not to compete. The plan to link Hamburg and Munich would also link seven major airports, providing significant relief for the overcrowded air lanes in West Germany, he said. Dreesbach argues that Japanese plans to build a maglev system should motivate West Germany to back its own superior technology.

The West Germans claim to be three or four years ahead of Japan, which they see as the main competitor in the potentially lucrative US market. Transrapid, which uses conventional magnets, last year set a speed record for a levitating train (with passengers) of 412 kilometres an hour.

Steven Dickman

Tokyo

JAPAN took a step towards developing a commercial railway line for a high-speed magnetically levitated (Maglev) train two weeks ago with a decision to locate a new 40–45 kilometre test track for the Maglev in Yamanashi Prefecture, about 100 kilometres west of Tokyo. The test track may later be extended to link Tokyo and Osaka for commercial operations. But before that many technological, economic and political hurdles have to be overcome.

The Yamanashi site was chosen by a Transport Ministry committee over two other possible sites, one in the northern island of Hokkaido, the other in the southern island of Kyushu where there is already a 7-km test track for the Maglev. The ministry will apply for ¥6,000 million (\$45 million) in fiscal year 1990 towards the cost of developing the track when budget requests are submitted at the end of this month. Total cost of the test track, trains and related facilities will be about ¥350,000 million and this is expected to be shared by the national government, the local Yamanashi government and one of the Japan Railway (JR) companies, JR Tokai, which was formed after the privatization of the Japan National Railways (JNR) in 1987.

The test line will be used for manned high-speed runs of up to 500 kilometres an hour and to test the Maglev on inclines and curves and in tunnels. So far the Maglev has been tested only on the straight 7-km track in Kyushu. But before it goes into commercial operation, the Railway Technical Research Institute which developed the train will have to find a way of shielding passengers from the strong magnetic field of the trains superconducting magnets. Watches, computers and heart-pacemakers are liable to stop when inside the institute's present test train (MU 002).

Apart from these technological hurdles, there are strong forces resisting plans of Maglev proponents to extend the line to Osaka and Tokyo. An Osaka-Tokyo Maglev could whisk passengers between the two cities in an hour instead of three hours by conventional bullet train. But this would threaten the commercial base of the existing bullet train lines (*Shinkansen*).

When JNR was privatized, the company had an accumulated debt of more than ¥25 million million (\$185,000 million), much of it resulting from the building of *Shinkansen*. The debt, which was passed to a semi-governmental company (the Japanese National Railways Settlement-of-account Enterprise) separate from the JR railway companies, has since risen to ¥27 million million, and interest payments on the debt alone amount to about

Australia plans high-speed train link

Sydney

AUSTRALIA'S plans for a Very Fast Train (VFT) link between Sydney and Melbourne have won the endorsement of Prime Minister Bob Hawke. In order to speed up the project, a Senate standing committee is to be established to examine the environmental, employment and development implications of VFT.

The train, described by Hawke as "the most important post-war development in Australia", will travel at top speeds of 350 kilometres an hour, providing the fastest commercial train service in the world. The line should be in service by 1996 at a cost of A\$4,500 million. Finance will come from private companies, including Broken Hill Pty Ltd, TNT, Elders IXL and the Japanese construction group Kumagai Gumi.

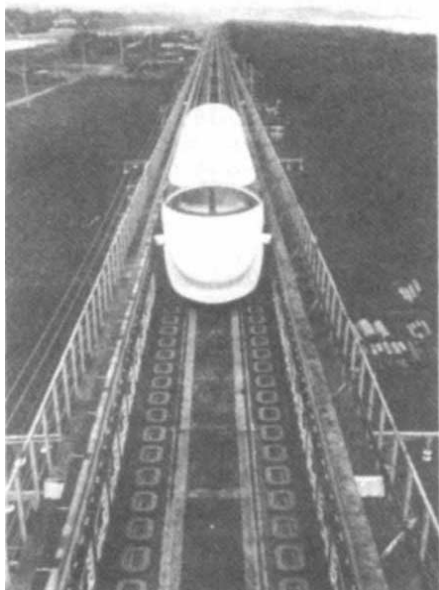
VFT will be important not just as a transport system but as a means of developing the Sydney-Melbourne corridor — a distance of about 1,000 kilometres, much

of it relatively isolated. According to Alan Castleman, chief executive of the VFT Joint Venture, there will be several types of service. Twelve express trains a day will travel direct from Sydney to Melbourne in just over three hours. From city centre to city centre, the travel time will be the same as by taking an aeroplane. Twelve daily trains will stop at 14 towns between the two major cities as well as providing an express service to Canberra. Journey times between Sydney and Canberra will be cut from four hours to one hour.

According to Castleman, VFT will be travelling at 350 kilometres an hour for most of its route. The French TGV Atlantic presently travels from Paris to Lyon at peak speeds of 300 kilometres an hour, and the TGV Nord planned for 1993 will travel at 320–330 kilometres an hour on the way to the Channel tunnel. Whether the VFT can maintain such speeds routinely over distances much greater than in France is being questioned. Tania Ewing

Maglev track

¥1 million million a year. The powerful Ministry of Finance is thus strongly opposed to any plan that could place a further burden on Japanese taxpayers. The cost of a Tokyo–Osaka Maglev line is variously estimated at between ¥3 million million and ¥10 million million.



Maglev, up to 500 kilometres an hour? (Railway Technical Research Institute.)

Nevertheless, there is strong support among the ruling Liberal Democratic Party for building a Maglev line and more *Shinkansen*. JNR's debt has been quietly forgotten by most of the Japanese public and there is a saying among politicians that if you get a *Shinkansen* (or Maglev) station located in your constituency you are assured a place in the Diet for life.

David Swinbanks

CONSERVATION

Biodiversity plan gets backing from NSF

Washington

LAST week, the US National Science Foundation (NSF) gave its backing to an ambitious plan to help preserve the Earth's biological diversity and halt what it calls "the most catastrophic loss of species in the last 65 million years". But the plan is likely to face an uphill struggle for funds in Congress.

In a report endorsed last week by the National Science Board entitled *Loss of biological diversity: a global crisis requiring international solutions*, NSF's committee on international science says that the foundation should increase funds for basic research in conservation biology, restoration ecology and environmental management, undertake an inventory of all living organisms, especially microorganisms, and increase support for research on biodiver-

SCIENTIFIC MISCONDUCT

Threats against witness

Washington

THREATS of legal action against a witness who testified at the most recent US congressional hearing on scientific misconduct have incurred the wrath of Congressman John Dingell, chairman of the House of Representatives subcommittee on oversight and investigations. As a staunch defender of the whistleblower in cases of misconduct, Dingell said he was "greatly concerned by what appear to be threats" and demanded evidence to justify them.

The incident was prompted by statements made by Dr Robert Sprague of the University of Illinois at Urbana-Champaign at a House of Representatives subcommittee hearing in June. Sprague, who exposed the fraudulent research of psychologist Stephen Breuning, said that the University of Pittsburgh tried to cover up the misconduct.

The vice-president and counsel for the medical and health care division of the University of Pittsburgh, George Huber, then wrote an angry letter to Sprague accusing him of making statements which were "not only inaccurate and untrue, but also slanderous and libelous". He said Sprague should stop making false statements about the university and should retract the inaccurate portions of his testimony, otherwise the university would take "whatever lawful corrective measures are necessary and appropriate, including the filing of legal action".

When Dingell heard of the letter, he responded with an equally angry letter to the president of the university, Wesley Posvar, expressing his astonishment and saying he believed that Sprague's testimony was "substantially accurate". A

week later, after a prompt review of the matter, Posvar informed Dingell that although there was no evidence of a cover-up, the university's investigation was not carried out with "sufficient zeal". He also wrote to Sprague, apologizing for the letter and commending him for his "vigilance and initiative on behalf of academic integrity".

Sprague says he criticized the university because its first three investigations focused only on research carried out by Breuning under subcontract to the University of Illinois and failed to scrutinize the research carried out for the University of Pittsburgh. No fraud was found in Breuning's research at the University of Pittsburgh until a fourth investigative committee reported on the matter in 1985, 18 months after Sprague's first accusations. By that time, Breuning had resigned, having admitted falsifying data related to his previous work with Sprague at the University of Illinois.

The university's counsel, Lewis Popper, who carried out the review, admits that it is "somewhat difficult to understand" why the university did not document and expose the fabrication of data at Pittsburgh and he says the university can be "legitimately criticized" for not doing so. Sprague's "impatience and ire" are understandable, he says. But he maintains that there is no evidence of an intentional cover-up. One reason why the committees might not have examined research at the University of Pittsburgh is that Sprague's initial allegations and proof focused on research carried out for the University of Illinois, says Popper. But Sprague says that the evidence supplied to the university was "equally devastating in both cases". For the investigations to ignore the research at the University of Pittsburgh was "mighty strange", he says. "I don't know how that could be explained by incompetence."

Popper also says that because Breuning had already resigned, it was difficult to investigate him and "arguably futile" to sanction him; that research was at an early stage and was terminated before he left; that the public health threat from fraudulent research at Pittsburgh was small compared with the risk from the research under subcontract to the University of Illinois, although Sprague disputes this; and that at that time there was less sensitivity and experience with investigating research misconduct that exists today. Because there is no evidence for a cover-up, Popper says, Sprague could be "legally vulnerable" if he repeated his accusations.

Sprague now says he will not repeat the allegations in future but will merely show his evidence and let others draw their own conclusions.

Christine McGourty

Christine McGourty

Privatization makes progress

London

THE two new British electricity generating companies, which will take over from the Central Electricity Generating Board (CEGB) when the industry is privatized, were launched last week. Lord Marshall, chairman of National Power, the larger of the two companies, said they relished the challenges of free market and that it was "wonderful" to move away from government control.

National Power will take over the lion's share of CEGB assets in preparation for its flotation, including responsibility for Britain's nuclear power stations. PowerGen, the smaller company chaired by Robert Malpas, which was formed to create competition, will be responsible for 26 per cent of the electricity supply. A third company, the National Grid Company, will run the transmission network. This will be a multi-million pound concern owned jointly by 12 distribution companies.

Many questions remain to be answered

about the timing of the £12,000-£13,000 million sell-off and the future of nuclear energy. Britain's ageing Magnox reactors have been withdrawn from the sale but National Power will still inherit 46 power stations, including five nuclear advanced gas-cooled reactors (AGRs), from the CEGB. Lord Marshall dismissed the suggestion that the AGRs would also be hived off and said the problems and costs of decommissioning them are "negligible" compared with Magnox. An intense dialogue continues, however, between the government and National Power about possible changes in future safety regulations. National Power maintains that it cannot be responsible for changes in regulations such as more rigorous safety procedures. The government has offered £1,000 million to pay for "unforeseen nuclear costs" and is to put an increased sum of £2,500 million before parliament this autumn to sweeten the nuclear pill.

The flotation timetable is in disarray

following the decision to remove the 26 Magnox reactors from the sale because of the potentially crippling costs of decommissioning them by 2002. The two companies were to be sold separately in autumn 1990 and spring 1991 but now a six-month delay may result in a joint flotation shortly before the next election.

At a press conference in London, Andy Roe, National Power's information officer, said they were still committed to the introduction of pressurized water reactors (PWRs). The only problems lay in the immense capital expenditure and the need for a guaranteed market. It is essential for the National Power and the generation boards to draw up contracts to reduce the risks of building PWRs. He added that the PWRs were needed for National Power to reach the required production level of non-fossil fuel laid out in the privatization bill. He also said the obligatory 600 MW of renewable fuel would be produced by the wind farm in Capel Cynon, South Wales.

Mr John Collier, chairman of the UK Atomic Energy Authority, said the nuclear industry would suffer from the government privatization programme. He said it was "unfortunate" that the government would be cutting research into fast reactors from £50 million to £10 million and would end support for the prototype fast reactor at Dounreay by 1994. In the authority's annual report, he also said the drop in funding for nuclear fusion was "very serious".

Ben Webb

ENVIRONMENT

Rain-forest canopy remains elusive ...

São Paulo

A NOVEL attempt to explore the upper canopy of the Amazon forest with a 'raft' suspended from a hot-air balloon was brought to a sudden halt last week when ten members of the French expedition were expelled from Brazil. The French group, headed by botanist Francis Hallé of the University of Montpellier II, had begun their activities without formal approval from the Brazilian government.

After a long wait for official approval of the project (see *Nature* 339, 569; 22 June 1989), Hallé and his colleagues arrived in Brazil with only tourist visas. They then test-flew their 'flying raft' near Manaus, in the state of Amazonas, without permission, angering the Ministry of Aeronautics.

Approval had been granted for the scientific part of the expedition from the Special Secretary for Science and Technology and the National Council for Scientific and Technological Development. But under Brazilian law, final approval could be granted only after study of the expedition proposals by the military.

The technicians were rash to set up the dirigible without waiting for final approval, admitted Gérard Xavier Kuhn, science and technology attaché at the French Embassy in Brasilia. Although Brazil's Ministry of Foreign Affairs was trying to play down the affair last week, the researchers will have to wait outside the country for approval for the expedition and for the proper visas.

Ricardo Bonalume Neto

... and there's no help from Washington

Washington

IN the United States, another novel project to reach the largely unexplored canopy of the tropical rain forest has ground to a temporary halt, but for a more conventional reason — the US Congress did not provide funds for it in the 1990 budget. Alan P. Smith, a scientist at the Smithsonian Institution, proposes to use a tall crane to reach the canopy. The crane could be set up even in inaccessible forest sites using a helicopter and, if required, moved from place to place. Precise and repeated access could be had to any point in the canopy within an 80-metre radius through the use of a manned gondola lowered from the crane.

Smith, who hopes to use the crane at the Smithsonian's research sites in Panama, says that the lack of such equipment means that carrying out research on the canopy is "like trying to do marine biology without a scuba system". Aerial ropeways have been constructed at several rain-forest sites but give easy access only to large branches, not to the branch tips where the flowers are, and where photosynthesis takes place.

A drawing of the crane, set in the midst of rain forest, appears in an exhibition at the Smithsonian which opened last week to celebrate the institution's tropical research and the completion of new research centre in Panama. But the crane itself, which requires an expenditure of \$300,000 plus construction costs, will have to wait until Congress is in a more generous mood.

Alun Anderson

INSERM

Monkey dispute

Paris

WHILE members of the French anti-vivisection group, Arche de Noé, face charges of theft, the 28 macaque monkeys stolen from a Lyons laboratory of the French national institute of health and medical research (INSERM) in May have still not been handed back to their owners (see *Nature* 339, 648; 1989). On Monday, INSERM's lawyer called for new charges of cruelty to be brought against the accused. Meanwhile a court of appeal was due last Tuesday, 22 August, to decide the fate of the monkeys, following the expiry of a custody order.

INSERM claims that identification tattoos removed by Arche de Noé involved the animals in unnecessary suffering and says that the wounds had not healed well. Arche de Noé has denied this. In a communiqué the group says that "the monkeys were operated on by a team of three veterinarians, under general anesthetic and in conditions of perfect asepsia".

"It is inconceivable", says the communiqué, "that our colleagues who risk their freedom for animals should be accused of acts of cruelty against these same animals."

Peter Coles

PARTICLE PHYSICS

SLC changes its tactics

San Francisco

AFTER a short-lived day in the sun during which it became the world's leading producer of elusive Z^0 particles, the Stanford Linear Collider (SLC) is now preparing to change tactics in order to avoid direct competition with a more powerful machine gearing up to speed at CERN, the 14-nation European scientific consortium based in Geneva.

For the SLC, which currently produces about 10 Z^0 's a day, the change has come too soon. By studying the Z^0 , one of the fundamental particles of the unified electroweak force, physicists will be able to pin down some of the poorly known parameters in their 'standard model' of elementary particles and forces. The original plan called for up to two years of dominance before the rival Large Electron-Positron (LEP) collider started up at CERN. Indeed, in the past few weeks, SLC scientists have announced the world's most accurate value for the mass of the Z^0 , as well as a new limit on the number of 'families' — sets of related quarks and leptons — into which elementary particles are classified.

But nagging technical difficulties have put the collider far behind schedule and dramatically reduced its time at the top.

Earlier this month, the more powerful LEP made its first Z^0 particles (see *Nature* **340**, 495; 17 August 1989), signalling a new era for the SLC. Once the European collider concludes its pilot run and is refined for full-scale efforts around the end of September, its California counterpart will have to relinquish its leadership role and instead try to fill niches. "It just really depends on how quickly CERN works its bugs out. After approximately October 1, it's going to be difficult to compete on a particle-by-particle basis", said SLC spokesman Michael Riordan. "We have to do special things that the nature of our detector allows us to do."

The first tactic involves electron beams, which collide to produce the Z^0 particles. While the LEP beams come together at a much higher rate than SLC's, and consequently produce many times more particles, the California facility employs a narrower beam that offers its own advantages. Because the particles the Z^0 decays into are extremely short-lived — lasting on the order of a trillionth of a second — many of them will decay while still within the wider beam used at CERN. Consequently, the LEP physicists will not be able to tell which came directly from Z^0 's and which are secondary creations, according to Riordan. The detection equipment needed to make such determinations should be installed at the SLC in early October.

For the longer term, beginning perhaps

in 1990, the SLC will use polarized electron beams, in which the electron spins are aligned with respect to the beam axis. By giving SLC physicists an extra degree of control over reactions that make Z^0 's, polarized beams permit the same level of accuracy to be achieved on certain measurements with 10 times fewer Z^0 's than

CERN. In measuring the Weinberg angle, the basic parameter of electroweak unification, SLC and CERN can thus compete on even terms.

In recent weeks, news accounts have focused on the well-known competition between the two facilities. By switching tactics, the SLC group hopes to play down the rivalry with CERN and emphasize ways for the two efforts to complement each other.

Robert Buder

EDUCATION REFORM

UK university charters stay the axe

London

THE resolution last week of a dispute over academic tenure at Aston University in Birmingham indicates that half of Britain's universities are still unable to dismiss tenured staff almost one year after the new education reform act ruled that they could.

Aston University wanted to impose compulsory retirement on 12 members of staff in the department of engineering. In response, the Association of University Teachers (AUT) threatened to take out an injunction to prevent the dismissals. The ensuing legal battle ended when the vice-chancellor of the supreme court, Nicholas Browne-Wilkinson, who was appointed to resolve the dispute, ruled that Aston's charter and statutes protect academic staff from compulsory redundancy unless the university has "good cause" to dismiss them. More than half of Britain's universities have similar charters.

Many universities are attempting to reduce staffing levels by voluntary redundancies and retirements to compensate for

financial cutbacks and falling student numbers in certain fields. But some universities are being forced to try more drastic steps to reduce costs. The new Education Reform Act allows the dismissal of tenured staff, but the charters under which the universities operate must first be rewritten. Five senior academics and lawyers have been asked by the Department of Education to look at individual charters to amend the clauses that guarantee tenure.

The new legislation is not retrospective, however, and any academic appointed or promoted before 20 November 1987, when the bill was published, will still be protected. David Packham, Aston's secretary and registrar, said that this was a serious flaw in the legislation and that some universities might suffer serious financial problems because they are unable to shed the academic posts they cannot afford. "The answer for some may be bankruptcy to save themselves and to worry about the consequences later," he said.

Ben Webb

SATELLITES

Indian launch vehicle grounded

New Delhi

INDIA'S Augmented Satellite Launch Vehicle (ASLV) has been grounded on the advice of an expert review panel after two successive failures. The panel has asked the Indian Space Research Organisation (ISRO) to modify the design of the rocket before attempting another flight. According to ISRO, the third ASLV flight incorporating the suggested changes will not take place for a year or more.

The panel suggested the design changes after a detailed investigation of the crash of ASLV during its second flight in July 1988. It concluded that ASLV is highly unstable (because of its high length-to-diameter ratio of 23) and that its control system is inadequate, particularly during its flight in the lower atmosphere where dynamic pressure is high. No control of the rocket is available during the critical phase between the burn-out of the two strap-on booster motors and the ignition of the first-stage motor. During the abortive ASLV flight, the boosters burnt out a little too soon and the period of "no

control" lasted for four seconds, during which the yaw angle rapidly increased. The increased load caused the vehicle to break up. These design inadequacies were not revealed in the first flight on 24 March 1987, as its failure was due to non-ignition of the first-stage motor.

ISRO has been asked to redesign the auto-pilot system and strap-on boosters, enhance the control margin and confirm them through additional simulation and destructive structural tests. All this work will be done by a new interdisciplinary design group. ISRO has admitted that the original design of ASLV failed to recognize the full impact of inadequate control during the critical phase flight or that of wind shears and gusts on the structural margins of safety.

ASLV is expected to be ISRO's workhorse for placing payloads up to 150 kilograms. But lessons learnt from it have already led to design modifications in the Polar Satellite Launch Vehicle (PSLV) which will place one-tonne payloads in polar orbit.

K. S. Jayaraman

Peer review (continued)

SIR—It is time that government funding agencies provided grants in support of the peer-review system. This crucial component of the scientific process can no longer be left to the goodwill of individuals because pressure of work means that reviewing has a low priority.

In Darwin's time, peer reviewing was easy. Most studies were descriptive or involved simple experiments. Today, reviewers and editors are no longer able to verify an experiment on a desk top because experiments are costly and time-consuming. For the same reasons, accurate and detailed communication of results by publication has become increasingly important.

At the same time, the pressures on individual scientists have caused the review process to become slower and less thorough. Prominent scientists may get 50 to 100 papers a year to review from colleagues and from journals. Some recent papers in mathematics have exceeded 100 pages and the effort required to review such a work exceeds the time available to most busy scientists.

My proposal is to provide for a rotating group of paid reviewers. Grants would be given for three months of work as a reviewer. They would be given on a lottery basis to individuals with a reasonable publication record, with the number of grants given proportional to the number of publications in each field. Individuals would be paid for their time and expenses. Each person could get the grant only once in, say, five years to avoid concentrating too much influence in a few hands. A listing of reviewers' specialities would be put into a database from which journals could pick those to whom they would send papers for review. As a limited supply of reviewers would be available because of limitations on funding, journals would be best served by sending to this group of paid reviewers only papers requiring extra attention, such as very long, very difficult or controversial papers. Most papers would still have to go to volunteers. But if the volume of papers needing review was reduced by this method, then volunteer reviewers might be able to do a more thorough job on the shorter or less controversial papers.

We have accepted the fact that many special tasks must be paid for in science. Whereas time used to be given voluntarily for editing scientific journals, most now have paid editorial assistants and even editors. Statisticians and other experts are paid for their advice and National Science

Foundation grant-review panels are funded. Why do we not see reviewing as a form of consultation? Only by paying for someone's time — and thus providing recognition for their work — are we likely to increase the level of effort people are willing to put into reviews.

A final consideration concerns the purpose of the paid review. There are two types of reviewer, the gatekeeper and the mentor. The gatekeeper tries to find fault and reject a substantial number of papers, in the name of professional standards, and this can be done quite quickly. If someone is being paid, he or she can perhaps take the time to act more as a mentor. In this capacity, a reviewer would check the equations, assist the author with clarity or logic, and spend some time really thinking about the paper. Such careful review may require reading related papers cited by the authors or performing some calculations. But if a study takes several months or a year to carry out and write up, a careful review should take a day or two. A mentor-type reviewer who does the job well may even end up as a coauthor on the paper, an added benefit of the position.

The question of funding for this system of grants of course arises. In fact, very little extra money would be required. Reviews are at present a 'hidden overhead', conducted in time paid for by grants given for other purposes. The costs of not having paid reviewers are very high. Papers are delayed for long periods waiting for reviews, and inadequate reviews do not help the authors to revise the paper for resubmission. Poorly written papers do not advance the field, and errors not detected before publication cause confusion and lower the reputation of science as a whole.

If 2,000 grants were awarded each year for three months of reviewing, then up to 200,000 papers could be reviewed thoroughly (assuming one to two days per review) at a cost of only \$30 million (salary plus overhead). The only cost is for direct labour, whereas normal research budgets include large amounts for expensive laboratory equipment and supplies. Spread over the huge budgets of the government funding agencies, this figure is a drop in the bucket.

CRAIG LOEHLE

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SIR—Although anonymous peer-reviewing has produced excellent results, the system has not been immune to insults and abuse (for example Craig Packer, *Nature* 340, 10; 1989). Here is a simple protocol that could bring fresh hope and restore the

respect the system deserves. I suggest that from now on the authors become anonymous and the reviewers come out of hiding. In this system, a journal editor would simply send a manuscript to a potential reviewer in the usual way but omitting the author's identity. The reviewer would have the option of remaining anonymous or having his or her identity revealed. Authors' identities should ideally have no bearing on the evaluation of a scientific piece of work.

GOBINDA SARKAR

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SIR—It is an indictment of the scientific community if one of us could even be suspected of being remotely instrumental in the precipitous action taken by Craig Packer's colleague (*Nature* 340, 10; 1989). In practice, most editors maintain the anonymity of all reviewers by distributing unidentifiable comments to authors and fellow reviewers. An equally fair system would evolve if anonymous manuscripts were peer-reviewed. Editors can request that copies of submissions for this purpose should have their title on the abstract/summary page without details of the author(s) or institution(s). The onus would then be on contributors to write their papers in a style that would make their ready identification less easy. One would then hope that personal attacks, most of which may indeed never come to light, would be eradicated at once.

K. N. TSQUAYE

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Names please

SIR—It has been decided to do a volume of omissions from the *Dictionary of National Biography* from the beginnings to 1985 (the 1981–85 supplement will be published next spring).

The new volume gives people an opportunity to suggest names of those they have looked up in the *DNB* and not found. This is the first time for a hundred years that such an opportunity has occurred. Previous volumes of the *DNB* have not been as generous to scientists as they have been to people from other walks of life. I would therefore be glad to hear from any of your readers with names to suggest.

C. S. NICHOLLS

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Correction

The author of the letter entitled "India's too costly imports" (*Nature* 340, 94; 1989) is Y. D. Sharma. □

Seeing the worst side of science

Andrew Tudor

Since the advent of the genre in the 1930s, horror movies have reflected public anxieties about science and technology. Through the years the images have changed.

"THE human mind will only stand so much — we're all a bit strange up here." So observes the aged butler in *Dr X*, First National's 1931 response to the huge horror-movie success of that year, *Frankenstein*. He was, of course, speaking of the dedicated band of scientists for whom he worked, their laboratory an isolated old mansion, their goal the creation of synthetic flesh. But he could as well have been describing horror-movie scientists at any time during the past 50 years, for, in this movie-made world, science is nearly always 'mad science'.

Along with supernatural and psychiatric disorder, mad science is one of the three most common sources of horror-movie monsters. Of the horror movies made between 1931 and 1984 that I have researched (almost 1,000 of them), more than a quarter posit science as one of the main causes of disaster. In so doing they tell us, if through a glass darkly, something about what people have found frightening over the years. In Tuan's elegant phrase, they map for us our "landscapes of fear", providing a fascinating insight into changing popular images of what is threatening about science and scientists. After all, if they are to make sense to their audience, they have to accord at some level with that audience's attitudes, prejudices and fears.

When you examine the genre's history, one general trend is immediately apparent. As the figure (overleaf) illustrates, there is a broad decline in the proportion of science-based horror movies after 1960, a decline coincident with the modern rise of the horror-movie psychotic. In the popular consciousness of the 1970s and 1980s it would seem, mad science has given way to a different kind of madness. Look more closely at the history, and it is also apparent that there are some periods

when the threat posed by individual mad scientists is less important than that posed by science itself. This is so in the 1950s and in the late 1970s and 1980s, reflecting the different evaluations of scientists' responsibility typically found in the movies of these years. With the growing public

by their overwhelming commitment to science. In the 1950s that changes; although it is still scientific knowledge that routinely causes mayhem, scientists themselves are more often portrayed as our saviours than as our executioners. Then, as the threat of horror-movie science recedes during the 1960s and 1970s, science is assimilated into a general fear of big institutions — multinational corporations, the state, the military — exploiters of scientific research who are represented as ruthlessly destroying our environment for the sake of profit and power.

British Film Institute

One fictional figure towers above all others in the classical development of horror-movie science. Baron Frankenstein, introduced in the spoken preface to the 1931 film as "a man of science who sought to create a man after his own image, without reckoning upon God", is the archetypal mad scientist. Devoted to the pursuit of knowledge at the expense of humane values, he and his successors (whether or not they bear his name) are permitted the equivocal comfort of defensible scientific motives. "Where should we be if nobody tried to find out what lies beyond?", asks Henry Frankenstein in *Frankenstein*, "Have you never wanted to look beyond the clouds

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

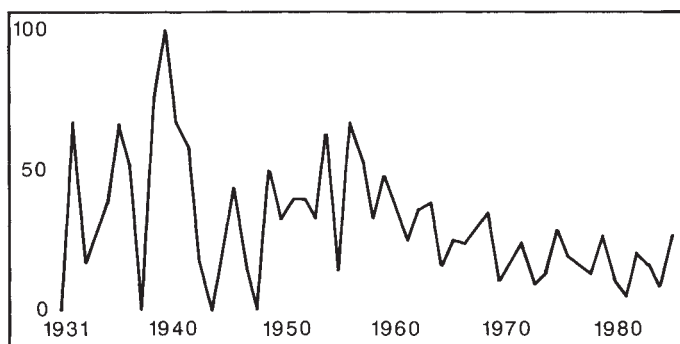
A character with "higher aspirations" — Dr von Niemann in *The Vampire Bat* (1933) seeks to create life in the laboratory but is corrupted by his obsession.

concern about atomic energy during the 1950s, the idea that scientists might unwittingly cause disaster becomes more common, a pattern that is repeated 20 years later when scientific side-effects are seen to give rise to pollution and ecological imbalance.

This issue of scientists' personal responsibility is central to the shifting pattern of the horror-movie vision of science. In the 1930s and 1940s, the actions of individual mad scientists were all-important — whether they were evil men pursuing their own ends or good men corrupted

and stars, to know what causes trees to bud and what changes darkness into light? But if you talk like that people call you crazy." Indeed.

At the other end of the decade in the exquisitely designed *Son of Frankenstein* (1939), where it rapidly becomes clear that the new Baron (played by Basil Rathbone) has inherited more than just an aquiline nose from his infamous father, the generic proclamation of scientific commitment comes in a letter that father has left for him: "If you, like me, burn with the irresistible desire to penetrate



Percentage of threats in horror movies caused by science.

the unknown, carry on. Even though the path is cruel and tortuous, carry on. Like every seeker after truth you will be hated, blasphemed, and condemned". Frankenstein, inspired by these words from the past and later shown his father's tomb on which someone has chalked "maker of monsters", takes up a symbolically burning torch and, using it as a pen, alters the crude epitaph to read "maker of men".

Such characters have higher aspirations (as one might guess from the way they so often fix their eyes on some distant off-screen point). Although most narratives do contrive to demonstrate that they are corrupted by their obsessive pursuit of knowledge, many movies also allow that relations between scientific and human interests may be genuinely problematic. Even someone as finally unredeemable as Dr von Niemann in *The Vampire Bat* (1933) is permitted an impassioned speech about the glories of science. "Mad?", he says, "Is one who has solved the secret of life to be considered mad? Life, created in the laboratory. No mere crystalline growth, but tissue, living, growing tissue that moves, pulsates, and demands food Think of it. I have lifted the veil. I have created life. Wrested the secret of life from life."

Of course, the fact that von Niemann finally comes to a sticky end (shot by his vengeful assistant) demonstrates his obsession to be truly insane. Yet the line dividing movie madness from genius is often less than clear; science, however threatening, also has its potential for good. So although existing authorities, such as the police or the local burghers, are the main narrative resource for dealing with those who stray from the path of scientific propriety, many of the period's monsters and their creators are finally destroyed not by those authorities but by each other, as if in implicit recognition of their joint sins. Only through

redeemed.

In the *Frankenstein* movies of the 1930s, sympathy is therefore as much with the Baron and with Karloff's anthropomorphic Creature as with the official forces of good. When the Creature in *Son of Frankenstein* studies his unprepossessing

reflection in the mirror, drawing Wolf von Frankenstein next to him to better make the comparison, we are more touched by his plight than by that of his erstwhile victims. And when the storm first ignites life in that lumbering frame in *Frankenstein*, it is the Baron's staring intensity at the scene's end which attracts all our involvement. "It's alive", he mutters, caught up, as we are, in the enormity of his achievement.

Thus it is that most of the mad-scientist movies of the early years, including *Dr Jekyll and Mr Hyde* (1931), *Dr X, Murders in the Rue Morgue* (1932) and *Frankenstein* itself, express at least qualified admiration for scientific inquiry. Fear of science is not straightforward here, for it is tempered by nascent ideas about the need for progress and the price of that need. As they struggle to create life, whether in the travesty humans of the *Frankenstein* tradition, or through the mechanical hearts of *The Walking Dead* (1936) and *The Man They Could Not Hang* (1939), or even in the ape-humans of *The Monster and the Girl* (1941) and *Dr Renault's Secret* (1946), these mad scientists reflect both the terror and the

promise of science.

In the final analysis, though, horror-movie science of this period is fundamentally disordering, revolving around inhuman ambitions which, by their very nature, give rise to inhuman threats. In seeking to interfere with the basic processes of life, science trespasses in areas quite properly forbidden to it — a belief occasionally given crude religious justification, though more often simply taken for granted. Dazzled and corrupted by the prospect of knowledge, scientists ignore the limitations implied by everyday values and loose their misconceived beasts upon an unsuspecting world. In this view scientists are powerfully threatening, their capacity to manipulate nature undoubted and all-embracing. As one character observes in *The Electric Man* (1941), "this theory of yours isn't science — it's black magic". Faced with such power, it is only by resort to violence and coercion that order can be restored and the pre-science world once more made secure.

In the 1930s and 1940s, then, the heyday of mad-science movies, scientists were either corrupted by their commitment or, less interestingly, they were evil men who bent science to satisfy their personal desires. But in the 1950s a new conceptualization of science-as-threat emerges. It had its precursors, of course. When the scientist of *The Invisible Ray* (1936), played by Boris Karloff, was driven insane and finally destroyed by Radium X, it was the first significant horror-movie allusion to the unexpected dangers of nuclear science. Twenty years later, with the likes of the giant ants of *Them!* (1954), the unseen radiation-consuming creature of *X the Unknown* (1956), the prehistoric monster liberated by nuclear explosion in

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Objects of pity — Boris Karloff's anthropomorphic Creature in *Frankenstein* (1931), and his creator, the Baron, self-sacrifice can they be inspire as much sympathy as do the official forces of good ranged against them.

Godzilla (1957), and the mutated molluscs of *The Monster that Challenged the World* (1957), the deliberate creations of the mad scientists of the 1930s have given way to the accidental products of nuclear testing.

There are two main aspects to this change. One derives from growing public awareness of the radiation risks of atomic energy. The other — though not unconnected — is more complex, extending and generalizing traditional horror-movie ambivalence about scientists' responsibility for their actions. In the 1950s scientists are no longer simply mad or bad — although they are dangerous to know; rather they have become the necessary instruments of progress. There is fear here, of course, the generalized fear that the engine of change is out of control, progress is not an unqualified force for good, and individual scientists — however well intentioned — are neither responsible for, nor in command of, the outcome of their researches.

Such a fear is central to the many films of the 1950s that focus on the monstrous side-effects of atomic energy. But, interestingly, it also permeates some of the period's more traditionally framed horror movies. Take *The Fly* (1958), for instance, one of the most commercially successful mad-science movies of the decade, and the first to be produced by a major studio (Fox) in both colour and CinemaScope. *The Fly* presupposes the familiar stereotype of the obsessed scientist whose precipitate haste to complete his research brings disaster. In the event, he is the victim. Testing his matter-transfer apparatus on himself, he unwittingly enters the machine in company with a fly, emerging with the fly's head and one claw-like hand, his own head and arm imposed on the insect. With no hope of reversing the process, he destroys his notes and his equipment, persuades his wife to cooperate in crushing his body in an industrial press, and leaves his brother to restore familial order with his wife and child.

This is a clear enough variation on the Frankenstein model. Yet where the scientist, André, differs from so many of his predecessors is in the absolute sympathy that the film accords him. He could hardly be described as 'mad' even to the limited

extent of, say, Wolf in *Son of Frankenstein*, and his disastrous haste is never motivated by a desire for self-aggrandizement. It is, rather, based on his wish to help humanity by exploiting the positive potential of his discovery. Unlike traditional mad scientists, he is not to blame; like all of us he is a victim of the modern age, turned into a creature of pathos as much as one of horror.

Thus, although the price of progress is certainly high in the films of these years, individual scientists are often relatively blameless. After all, this is the era in which the 'scientific expert' is most effective in dealing with a wide variety of movie

(1956), is researching nutrients in the hope of resolving the world's food problems, work which kills him and his staff as well as releasing an overgrown spider into the local community. Typically, only a combination of scientific expertise and military strength finally eliminates the threat (the hapless arachnid is finished off with napalm). Such films are notable in that their scientific researchers are neither obsessed nor evil; they are simply unlucky. In trying to improve the lot of humanity they have the misfortune to destroy themselves or others. In this movie world, science no longer corrupts its practitioners; but it is, by its very

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nature, a risky enterprise, and for that reason it can often be dangerous.

Unsurprisingly, the Frankenstein tradition of crazed and corrupted scientists does not sit easily in the horror-movie world of the 1950s. There are some mad scientists, but they are often more humorous than horrific. Just the title of *I Was a Teenage Frankenstein* (1957) suggests the lower depths of invention, although it does boast an immortal line delivered to his teenage creation by the film's mad medico: "I know you've got a civil tongue in your head because I sewed it there myself". Only Hammer Films (beginning with *The Curse of Frankenstein* in 1957 and continuing throughout the 1960s) really sought to extend the traditional Frankenstein conception. In so doing, they shifted its emphasis away from the corrupting potential of science and towards a more simplistically evil characterization of the mad scientist.

In the 1950s, the inhuman ambitions of individual scientists are far less to the fore than hitherto, and the familiar moral ambiguity of the Frankenstein tradition is painted onto a much larger canvas. This science is no longer the quasi-magical activity of the 1930s, tucked away in old houses and gothic castles; it is more prosaic and more all-embracing. Science has become a constitutive part of our everyday world, its admired and feared exponents harbingers of both progress and disaster, its most common threat — radiation — unseen, but a potential invader of every corner of our lives. Perhaps, then, the most symptomatic image of this period is not *Godzilla* laying waste to Tokyo or

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

An early allusion to the dangers of nuclear science — Boris Karloff (left) plays a scientist driven insane and finally destroyed by Radium X in *The Invisible Ray* (1936).

threats, not simply those created by science itself. Specialist scientific knowledge is recognized as both dangerous and essential, scientists' expertise irreplaceable: Professor Quatermass may be indirectly responsible for the invading space monster of *The Quatermass Experiment* (1955), but he is also the only person who can destroy it. This double-edged evaluation of scientific expertise was not new, of course. But it was given additional force in the 1950s, perhaps most clearly in those radiation-obsessed horror movies whose ancestor was *The Invisible Ray* of 1936. There is an irony here, a definite discrepancy between the enormity of the nuclear threat and the credibility routinely afforded to 1950s' science. Perhaps, as so many have argued, our popular culture really was trying to teach us "to stop worrying and love the Bomb".

Many films of the 1950s feature scientists whose pursuit of scientific goals is admirably motivated. Deemer, for example, the biochemist in *Tarantula*

the giant ants of *Them!* struggling for their lives in a city sewer. It is the eponymous hero of *The Incredible Shrinking Man* (1957), dwindling victim of accidental irradiation, desperately seeking only to survive in a universe which no longer offers him (or us) a secure and central place.

As this assimilation of science into the everyday world extends through the next three decades, scientific research is increasingly represented as no more than a variously effective means for pursuing variously lunatic ends. Whether one seeks to rule the world by reanimating refrigerated Nazi soldiers, as in *The Frozen Dead* (1967), or merely to capture the heroine's heart by mutating into a jellyfish and killing off the romantic opposition, as in *Sting of Death* (1968), science largely serves as an unexplicated instrument of evil. Although many favourite mad-science enterprises still remain credible — solving the secret of life, preserving youth, fostering mutations — the concept of science to which they relate has lost whatever distinctiveness it once had. Science in itself is no longer intrinsically frightening, and scientists, whichever side they are on, are much diminished. Faith in the expert scientist as a bastion against all manner of threats has virtually disappeared by the 1970s, a decline which is, finally, the most characteristic feature of this last phase in the evolution of horror-movie science. By the late 1970s, even the most explicitly science-orientated of horror movies actually pay little attention to science.

Thus, although science may be necessary to precipitate the kind of ecological disasters common in modern horror movies, as in *The Crazies* (1978), where a biological weapon is accidentally released into a town's water supply, it is more often the military, the state or the big corporations who are seen to be guilty. What was once a consequence of scientific over-ambition or individual misuse of knowledge is now integral to the activities of the very authorities who used to be central to our defence. This presumed conspiracy between the institutions of science, state, military and industry is a thread running through many modern horror movies, not simply those which make overt reference to science. In *Piranha* (1978) the rampaging fish have been created (by science) as weapons for use in Vietnam. Accidentally released into a local waterway, their existence the subject of a military cover-up, they can only be destroyed by polluting the water with chemical waste from a convenient factory. If, that is, they are destroyed — for, like most such films, *Piranha* keeps its options open.

Generally, of course, horror-movie pollution has the opposite effect. The spreading mutations of *Prophecy* (1979) are a consequence of mercury poisoning from a local lumber mill; the unnatural

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Science out of control — after accidentally and irreversibly transforming himself into a half insect/half human monster in *The Fly* (1958), the scientist persuades his wife to end the horror by crushing his body in an industrial press.

nasties of *Humanoids from the Deep* (1980) are the accidental product of experiments to increase the yield of local salmon; and the giant reptile of *Alligator* (1982) — of which its writer, John Sayles, revealingly observes, “my original idea was that the alligator eats its way through the whole socio-economic system” — mutates as a result of consuming animal remains from a big corporation's hormone experiments.

Science functions strictly instrumentally in modern horror movies. If mad-scientist films in the 1930s were about knowledge and its dangers, those of the 1980s are about something else. They postulate a world under threat of imminent destruction, individuals open to horrifying metamorphoses, and established authorities either helpless in the face of catastrophe or corruptly involved in its genesis. Science here is just one way in which power can be exerted over a desperately resisting population; scientists just one repressive tool among many. Science has become the servant of other interests, and the once visionary faith of the mad scientist has now been reduced to a fast-crumbing defence against apocalyptic social and physical collapse.

Perhaps it is not surprising. In a fictional world in which people can be routinely and inexplicably transformed into

psychotics or zombies, a belief in science seems peculiarly inappropriate. Throughout the modern genre, expertise — meat and drink to science-based horror movies — has been devalued. As civilization collapses all around them in *Dawn of the Dead* (1980), a TV interviewer talks to a bearded and black-eye-patched scientist, an echo, perhaps, of Herman Kahn and the days of “thinking the unthinkable”. The scientist, despairing and exhausted in the face of an intractable situation, still asserts the faith of experts everywhere. “We’ve got to remain rational”, he says, “logical, logical, logical . . .”. He sinks into hypnotic repetition of “logical” and the interviewer's voice rises above his: “Scientists always think in those kinda’ terms. It doesn’t work that way. That’s not how people really are”.

As ever in the horror movie, the scientist doesn’t even hear him: “. . . logical”, he continues, “we have no choice. It has to be that way. It’s that, or the end”. It is one measure of the pessimism of the horror movie's modern vision of science that “the end” is exactly what it turns out to be. □

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High-energy physics needs thought

Proper excitement that CERN may answer many important questions should be moderated by some deliberate plan for true global collaboration in high-energy physics.

BIG-science people are a patient lot, and have to be. Projects costing \$1,000 million (such as the Hubble Space Telescope) or more (the Superconducting Super Collider, SSC, now to be built in Texas, for example) are necessarily years in gestation. Those who conceive of grand projects may often have died or retired by the time their dreams come true. The difficulties for younger people seeking to make their way in a competitive world on the basis of published contributions are even more acute. Often there may be nothing to give to prospective employers, or fellowship committees, except letters of recommendation from more senior people.

Much has been said about the sociology of these circumstances. That the originators of projects may not be those who see them through, and who reap the rewards of discovery, engenders a sense of teamwork and a seemingly sense that discovery is (in Vannevar Bush's evocative phrase) an endless frontier. The minuses are different. The teams are often huge — so large that young people may be lost in them, or may find that success hangs on qualities not strictly those of science, managerial skill, for example. The other side of that coin is that young people also learn a great deal when working as members of large big-science teams.

There is also a sense in which large projects engender impatience. When people know that it will take a decade to complete a project, they are understandably eager to get on with it. But megaprojects almost always require that some government or governments should have learned to temper impatience with a proper sense of responsibility, persuading politicians that a project for which funds are sought is a significant improvement on what exists already, and is likely to yield a substantial harvest of discovery.

These platitudes are prompted by the appearance in *Physical Review Letters* of two articles describing the accumulated measurements at Fermilab and SLAC (the Stanford Linear Accelerator Center) of Z^0 bosons, likely in the next few months to be produced in great abundance at CERN (the European High-Energy Physics Laboratory at Geneva). It makes sense that the data should be published now, if only as a benchmark against which CERN can calibrate its own measurements. For what it is worth, the measurements described by Fermilab and SLAC

(63, 720 and 724 respectively; 1989) agree within the experimental uncertainties both with earlier data from CERN and with the prediction of the standard theory due to Salam and Weinberg. Each article is signed by roughly 200 people; those interested may count them.

The SLAC article, interestingly, does not include the name of W.F. Panovsky, director at SLAC for many years until his retirement three years ago. That illustrates the selflessness with which the originators of important projects create them for the benefit of others: Panovsky was the driving force behind the creation at SLAC of the machine called the Stanford Linear Collider (SLC) with which SLAC's Z^0 bosons have been made. The idea is that pulses of electrons and positrons from SLAC's main electron accelerator are separated magnetically, then focused by means of magnets in roughly semicircular arcs about a kilometre in circumference and finally made to collide.

The technique is among the most exacting so far practised even in high-energy physics. Each pulse of electrons consists of 10,000 million particles. The trick is to arrange that each pair of pulses hits the same patch of geometrical space, roughly 3 micrometres in diameter, at virtually the same time. It is understandable that it should have taken a little longer than first planned — two years extra, by some estimates — to get the counter-circulating pulses into the planned shape. No doubt everybody working on the project has learned a great deal from the experience, the virtues of patience particularly. As the only particle accelerator in which bunches of particles are fired at each other as if they were bullets, SLC will also provide useful design experience for machines not yet built. The Soviet Union has already decided in principle to build such a machine at Serpukhov, while another on the same lines is spoken of as a successor to the advanced version of the Large Electron-Positron collider now producing Z^0 particles at CERN.

But there is a delicate irony in all this. If SLC had indeed functioned as designed when first commissioned, it would by now have generated some thousands of unambiguously identified Z^0 particles and CERN would have been deprived of the opportunity to produce the definitive data on the properties of these particles, now expected in the next few months. That does not mean that the money spent on

LEP would have been wasted — there are plenty of other things to do with LEP (see *Nature* **340**, 277; 1989). But the gilt would have gone from the gingerbread. And while it would be wrong to say that SLC was built to steal CERN's thunder, the sense that this might happen helped to fortify the resolve of SLAC — and helped to persuade the US Congress to approve the money.

There is also a moral. Nobody will deny that competition has a place in science, or that the competition to build more effective accelerators and to commission them on time has been an efficient spur to the development of high-energy physics. But with even (perhaps, given the federal deficit, one should say "especially") the US Congress sucking its teeth over the prospective cost of SSC, the time has come to ask what can be done to make competition more efficient, or less wasteful of resources. There is, in other words, a need that the development of new machines should be invested with a still greater sense of deliberation, however painful the present slow process of winning approval for a new project may be.

This, sadly, is where the high-energy physics communities on both sides of the Atlantic become evasive. The obvious end-point in the field is global international collaboration. So much seems generally to be accepted by high-energy physicists in the United States and Europe, but with a reservation reminiscent of that applied by St Augustine to his prayer that God should make him chaste — "but not yet". The accelerator after next is when the time will be ripe, is what the people say.

That cannot continue to be a respectable riposte. Even as things are, the reality of transatlantic collaboration in the field is substantial, as is that between Japan and European and US laboratories. Why not formalize these arrangements, which would require some kind of general consensus about the design of the next generation of accelerators and agreement about their siting? People's reservations seem usually to be stimulated by the calculation that, then, there would be only one machine being built at any time. That need not necessarily be the case, and only one machine would be better than none, the outcome if the world's taxpayers turn sour. That might mean that patience would have to be more widely practised. Would that matter?

John Maddox

Broad minded on narrow spikes

Robert Meech

IT IS natural to suppose that the longer and larger the action potential that arrives at a chemical synapse, the greater the rise in intracellular Ca^{2+} and the more neurotransmitter that it causes to be released. Just such a mechanism may contribute to the analgesic action of opioids in vertebrates¹ and to behavioural sensitization in *Aplysia*². But on page 636 of this issue³, A. N. Spencer, J. Przysiecki and J. Acosta-Urquidí report that at the neuromuscular junction of the jellyfish *Polyorchis penicillatus* precisely the reverse is true: short action potentials produce a larger postsynaptic event than longer ones. This is not a specialization of simple systems like jellyfish, for the explanation is a general one with implications for nervous systems as complex as those of the insects or mammals.

In 1967, Katz and Miledi⁴ used squid axon giant synapses treated with tetrodotoxin to show that transmitter release depends on the amplitude and duration of the depolarization at the presynaptic terminal. In depolarized squid axons, calcium activation develops so slowly that the rise in internal Ca^{2+} follows much the same course as the increase in potassium conductance⁵. Hence, when Llinás, Steinberg and Walton⁶ reconstructed the events during normal synaptic transmission, they showed that the calcium responsible for transmitter release enters the terminal during the repolarizing phase of the action potential. Accordingly, the slower the rate of repolarization, the greater the calcium influx at the presynaptic terminal and the larger the associated postsynaptic response. This has been confirmed experimentally in vertebrates by Lin and Faber⁷

using synapses on the Mauthner cell of the goldfish.

Prolonged action potentials are not always associated with a greater rise in intracellular Ca^{2+} , however. In molluscan neurons, successive impulses in a train of action potentials grow in duration as a result of the cumulative depression of voltage-dependent potassium currents⁸. Use of the calcium-sensitive dye arsenazo III injected into the giant neurons of *Aplysia* has shown that the action potentials may broaden by as much as 50 per cent with no apparent change in calcium influx⁹. This is because the pronounced shoulder on the falling phase of each of the prolonged action potentials is near 0 mV. Most calcium channels are closed at this voltage in these cells and so the prolongation has little effect on the change in intracellular Ca^{2+} measured by arsenazo.

Compared with *Aplysia*, the position of the calcium activation curve in squid¹⁰ and *Polyorchis*³ is shifted along the voltage axis so that there is a substantial inward current at 0 mV; this is illustrated for the *Polyorchis* motor neuron, (a in the figure) which shows a maximum inward current at about +10 mV. As a result, prolongation is most effective with relatively low amplitude action potentials. This is because the driving force on the calcium ion decreases as the membrane potential approaches the calcium equilibrium potential and, although the calcium channels are open, calcium entry becomes smaller not greater. With a large amplitude action potential it is not until the membrane begins to repolarize that the driving force becomes sufficient to promote calcium entry.

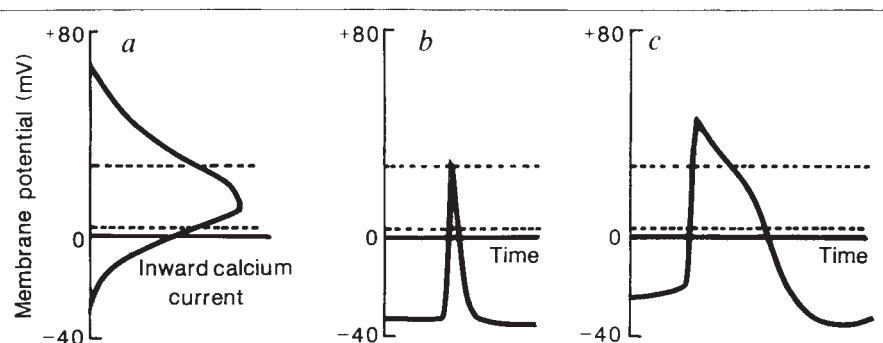
The *Polyorchis* preparation differs from

the squid synapse in that its action potential is sufficiently prolonged for calcium channel inactivation to become significant. In *Polyorchis*, the entire overshoot of the shorter action potential is associated with a near maximum inward calcium current (b in the figure) but the peak of the longer action potential is out of this range (c in the figure) and so there is relatively little calcium entry until the later stages of repolarization. Spencer *et al.* suggest that during the early stages of the action potential many calcium channels are inactivated and become unavailable. As a result, calcium enters the cytoplasm at a low rate and much of it is sequestered by cytoplasmic calcium buffers. With entry and sequestration rates nearly the same, there would be little change in Ca^{2+} and little release of transmitter, just as Spencer *et al.* observe.

Although it seems that the basic mechanism of synaptic transmission may be universal the performance of each synapse, whether from goldfish, squid or jellyfish, depends on the shape of the presynaptic action potential, the voltage dependence and kinetics of the presynaptic calcium channel, and the kinetics of cellular regulation. More preparations are needed to evaluate the influence of these different factors. The *Polyorchis* preparation developed by Spencer and colleagues has the advantage of good electrical access to the synaptic sites and so further dissection of the presynaptic currents may be possible. But it is just one such preparation identified in jellyfish. Others are the neuromuscular synapse of *Aglantha*¹¹, with its dual system of action potentials, and the bidirectional synapse of *Cyanea*¹². Researchers with patrons farsighted enough to support these ventures into the realms of the jellyfish have returned with great bounties. The advantages of using the simple nervous system of the jellyfish for experimentation are more than just travellers' tales. □

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a, Step changes in membrane potential at the presynaptic terminal produce a transient inward calcium current across the terminal membrane. In this representation of data from *Polyorchis* motor neurons³ the peak of the inward calcium current is shown on the horizontal axis with the membrane potential plotted vertically. The range of membrane potentials at which the calcium current is greater than 75 per cent of maximum lies between the dotted lines. In *Aplysia* neurons the relationship between inward calcium current and membrane potential is displaced to more positive voltages (upward in the figure); in squid axons the relationship is displaced downward. b, c Intracellularly recorded action potentials from *Polyorchis*³ showing that the calcium influx is near maximum for almost the entire time that the smaller shorter action potential (b) overshoots 0 mV, whereas the longer action potential (c) enters this range only after a considerable delay.

Cosmic merger mania

E.S. Phinney

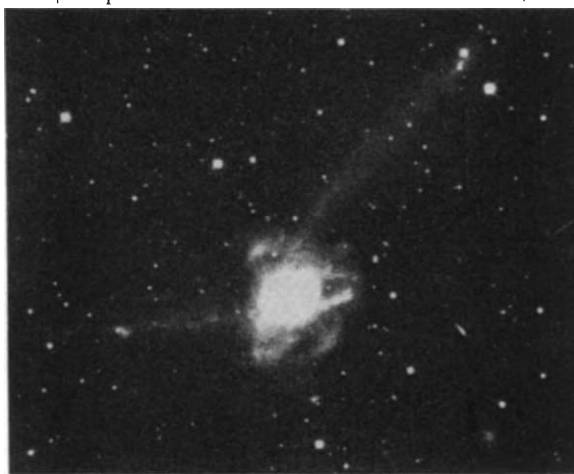
GALAXIES have sometimes been called island universes. But just as islands grow, crumble and move about on geological timescales, so galaxies grow, move and merge with one another on cosmological timescales. The *Dynamics and Interactions of Galaxies* was the avowed topic of a recent conference*, but the real theme was the question: to what extent are the properties of the galaxies we see today a result of the collisions and subsequent mergers of earlier generations of galaxies?

In the early 1970s, many people would have answered "entirely". A popular view was that small clusters of stars formed at high redshift z (relatively soon after the Big Bang, perhaps as early as $z \approx 10^3$), and repeatedly merged to form galaxies and clusters of galaxies. Unfortunately galaxies are clearly defined entities, not part of a self-similar hierarchy. So it later became popular to argue that galaxies sprang almost fully formed from the coherent collapse of high-density regions of the expanding Universe.

Throughout this dark age, a few — notably Alar Toomre¹ — maintained that mergers of galaxies must be important. Fewer than 1 per cent of the galaxies visible in our snapshot of the Universe are involved in the violent interactions that lead to coalescence, but those stages are shortlived. The actual number of galaxies that must have had such interactions is larger by the ratio of the age of the Universe to the lifetime of the evidence. Although no one has yet made a careful estimate using models of the interactions in a complete census of galaxies, the impression is that at least 10 per cent, and possibly a majority, of galaxies must have merged with other galaxies of similar size in the most recent half of the age of the Universe. Mergers were, if anything, more frequent in the past.

Small galaxies are much more numerous than big ones. Almost inevitably, every large spiral galaxy must have captured many smaller galaxies. Indeed, a steady dribble of gas is needed to maintain the open spiral arms so characteristic of gas-rich spiral galaxies (A. Toomre, Massachusetts Institute of Technology). The rings of gas orbiting the poles of some spiral galaxies are convincingly interpreted as the remains of the capture of a particularly loosely bound companion (L. Sparke, Univ. Wisconsin).

The merger of two bright collections of stars can only lead to a still brighter collection (though in making comparisons, one must be careful to account for the subsequent evolution of younger stars present and perhaps created during the merger). The remnants of mergers should thus be found among known galaxies. Obvious candidates are elliptical galaxies and the piles of stars (bulges and spheroids) surrounding the disks of spiral galaxies. Improved instrumentation has uncovered



The merger candidate NGC 7252. The velocities of the two tails (the longer one is at least 4 Milky Way diameters in length) suggest that this galaxy is the result of a merger, begun about 10^9 years ago, of two disk galaxies¹¹. Its spectrum shows younger stars than are found in a normal elliptical galaxy, probably created from gas compressed in the violent merger. The radial brightness profile and velocity dispersion are characteristic of an elliptical galaxy. In another 10^9 years, the young stars will have faded, the tails have dissipated and the loops have phase-mixed away. The result will probably be indistinguishable from an elliptical galaxy. (Photograph by F. Schweizer with the 4-m telescope at Cerro Tololo, Chile.)

subtle fossil evidence supporting this view. K. Freeman (Mount Stromlo Obs.) reviewed the curious properties of our own Milky Way. One of the most suggestive is the apparent absence of stars near the Sun with angular momentum per unit mass l larger than the Sun's, $l_\odot$. There are many stars with $l < -l_\odot$, but few with $l > l_\odot$. This anisotropy could be understood if the high-velocity stars were the disrupted constituents of small satellite galaxies captured by the Milky Way. Those satellites with orbits in the same sense as the rotation of our disk would be quickly pulled into the plane of the disk and their orbit circularized², so their disruption would leave no stars on significantly non-circular orbits. Satellites on retrograde orbits, by contrast, would interact only weakly with the disk, and their decaying orbits could remain eccentric. They would leave a population of retrograde high-velocity stars like that observed. B. Carney (Univ. North Carolina) pointed out that if such

satellite captures were common in the early history of our Galaxy, the energy deposited could increase the scale height of the stars then in the disk so much that we would not now identify them as part of the disk. This might explain the embarrassing paucity of old low-metallicity stars in the disk (the famous 'G dwarf problem'); indeed the missing stars appear to be present in the so-called thick-disk population.

Elliptical galaxies were once defined as featureless piles of stars. It now seems that most of the bright ones contain faint ripples, crosses and plumes of starlight (which correlate with spectroscopic evidence for young stars; F. Schweizer, Carnegie Inst.), all clearly evidence of recent capture of a disk galaxy (J.-L. Prieur, European Southern Obs.). Their faint disks and box-shaped isophotes (R. Bender, Landessternwarte Heidelberg), the peculiar twists and reversals of the streaming of their stars (S. Wagner, Landessternwarte Heidelberg), and their warped disks of gas (T. de Zeeuw, Calteen) may also be evidence of cannibalism.

Giant elliptical galaxies at the centres of galaxy clusters have long been suspected of growing by capture of cluster members. Estimates of the rate of capture in present clusters indicate, however, that no more than about a third of the stars in these central galaxies can be accounted for in this way (T. Lauer, Princeton Univ.; S. Tremaine, Canadian Inst. Theoretical Astrophysics). Fortunately, mergers of clusters of galaxies seem to be quite common. In such mergers, the heaviest galaxy of each cluster would tend to sink to the bottom of the combined potential well and

merge with its competitor. Binary giant galaxies are seen in about a fifth of clusters and are expected to last for about a fifth the age of the Universe (Tremaine), so giant galaxies, like giant companies, may grow mainly by merging with other giants, not by capturing small fry.

About 1 per cent of galaxies lie in very compact groups with 2 or 3 others. The galaxies in these compact groups will collide and merge in a time difficult to estimate, but probably of the order of one-fifth of the age of the Universe (S. White, Steward Obs.). Numerical simulations of such mergers, now quite realistic³, show that the result of merging two or more disk galaxies is an excellent facsimile of an elliptical galaxy (J. Barnes, Inst. Advanced Study; P. Quinn, Mount Stromlo Obs.).

The simulations have also mitigated the force of some long-standing objections to mergers⁴. The orbital energy of the merging galaxies is not shared equally by all the stars, nor does the merger mix stars

* Heidelberg Conference on Dynamics and Interactions of Galaxies, 29 May–2 June 1989, organized by SFB 328, University of Heidelberg.

randomly: dynamical friction gives the orbital energy primarily to loosely bound stars and dark halo particles, ejecting some. Stars in the tightly bound cores of the pre-merger galaxies end up in the more tightly bound cores of the final system. Mergers thus preserve correlations of the properties of stars with their binding energies (M. Franx, Harvard Univ.).

Results from the Infrared Astronomical Satellite (IRAS) have recently drawn attention to the merger process itself. The easily simulated mergers of galaxies composed largely of stars are rather dull. Excitement is provided by gas, which can radiate, form stars and transport itself by forces stronger than that of gravity. The brightest galaxies in the infrared sky seem to be the products of recent violent interactions (R. Joseph, Imperial College; U. Klaas, Max Planck Inst.). Observations of molecular transitions at millimetre wavelengths with new interferometers show that the gas has been concentrated toward the centre of such galaxies⁵, and heroic simulations have given us some understanding of how this occurs (M. Noguchi, Tokyo Univ.; F. Combes, Paris Obs.; L. Hernquist, Inst. Advanced Study). On a scale another 10^8 -times smaller may lurk a central black hole. Ideas on how gas may lose enough angular momentum to reach it are still qualitative (E.S.P.; ref. 6), yet reach it the gas seems sometimes to do. Many, perhaps most, quasars and powerful radio galaxies show signs of recent interactions (A. Stockton, Inst. Astronomy, Hawaii; T. Heckman, Univ. Maryland). But few of the less dramatically active galaxies, like Seyferts, are obviously connected with interactions or mergers (J. van der Hulst, Groningen Univ.).

At the meeting, then, emerged a picture of the effects of collisions between present-day galaxies. Still lacking is a

convincing calibration of the rates of collisions and the lifetimes of the various observed pre- and post-merger phases. Without this, we cannot say whether the present forms of galaxies were largely determined by the time the Universe was a tenth of its present age, or whether most galaxies have had their structure altered by more recent mergers. As G. Efstathiou (Oxford Univ.) emphasized in the opening lecture, the cold dark matter model, our only theory of galaxy formation still both viable and predictive, suggests the latter⁷. There are intriguing spectroscopic hints that many galaxies at a redshift of 0.5, when the Universe was about half its present age, were very different from galaxies today⁸⁻¹⁰. The much-delayed Space Telescope will tell us whether their shapes and sizes are different too. Yet proving that mergers are the cause, will require completing the tedious task of measuring the rates and lifetimes of galaxy-galaxy reactions. □

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PALAEONTOLOGY

A backbone for the vertebrates

Henry Gee

COMPARATIVE zoology is experiencing a quiet revolution. For the past 20 years, a view of chordate origins has been emerging which combines classical anatomy with modern cladistic theory. In the latest¹ of a long line of papers, R.P.S. Jefferies, with A.J. Craske, describes the systematic position of a fossil invertebrate called *Barrandeocarpus norvegicus* (Fig. 1) from the Upper Ordovician of Norway. Many researchers consider carapoids, the group to which *Barrandeocarpus* belongs, as a long-extinct group of echinoderms. But following earlier theoretical work², Jefferies and his co-workers have thrust the honour of vertebrate ancestry on this obscure group³, calling them calcichordates⁴ — that is, chordates with an endoskeleton

of calcite, like echinoderms.

Arguably the most widely accepted view of chordate ancestry stems from Garstang^{5,6}, who believed that the distinctive 'tadpole' larva of tunicates was an innovation, interpolated between ciliated larva and sessile adult⁷. Vertebrates would have evolved from tadpole larvae by pedomorphosis⁸. Garstang's ideas were based on a meticulous organization of the data in what might almost be termed a cladistic way (see below). This rigour might explain why his ideas have stood the test of time.

Jefferies, on the other hand, believes that adult ancestral chordates were free-living, anatomically very much like tunicate tadpole larvae, but with a calcite

endoskeleton. The sessile stage came later — a specialization of tunicates rather than a secondarily lost feature of chordates.

Although Jefferies can trace the order in which chordate features were acquired in a way that is consistent with chordate structure, he is hampered, like Garstang, by the lack of fossil evidence for the conceptual leap from the calcichordate to the recognizably vertebrate condition. The most advanced vertebrate ancestor known

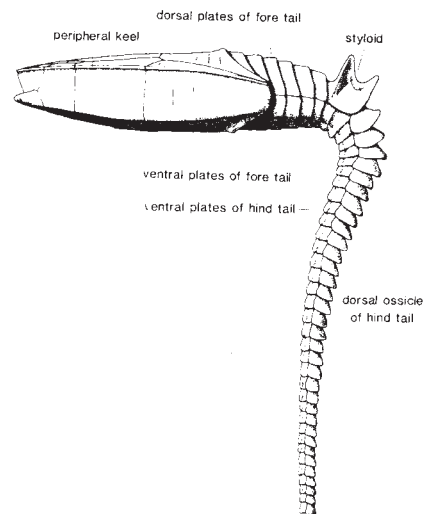


FIG. 1 Reconstruction of the mitrate *Barrandeocarpus norvegicus* in left lateral view, by A.J. Craske (from ref. 1). Jefferies (ref. 3) interprets the flexible, segmented tail region as being based around a notochord. The head region contains the brain and viscera, and the mouth is at the front (left of the figure).

among calcichordates is still a tunicate tadpole larva supported by calcite. No modern chordate has a calcite skeleton, and a radical revision of the body plan would necessarily follow its loss. This revision would be consistent with anatomical observation but rests on an extended chain of reasoning which at least one observer believes is stretched too far for credulity⁹, despite the minute attention to detail at each stage.

What marks Jefferies' ideas out from competing hypotheses is the explicit use of cladistics — in particular, the way in which he applies cladistic methods to classifying fossil groups. Cladistics, an analytical method for reconstructing phylogenies, is based on identifying groups defined by sets of derived characters shared by all members. These groups are termed monophyletic¹⁰. But groups based on primitive characters, or the absence of derived characters, are termed paraphyletic because they cannot be defined uniquely on the basis of derived traits.

Coping with paraphyly is the thorniest problem in systematic palaeontology. It is relatively easy to avoid paraphyly when classifying extant organisms, but how can fossils be organized in a phylogenetically useful way? Some have sought to get round the problem by asserting that fossils are never informative enough to overturn

a phylogeny constructed using data from extant taxa¹¹⁻¹³. In a News and Views article last year¹⁴, I discussed how fossil species along a transformation series could be used to overturn neontologically based cladograms in certain circumstances¹⁵. But the transformation series is a paraphyletic concept derived from traditional, synthetic ideas which can be expressed in cladistic terms only by a careful choice of characters used to justify existing ideas of relationship¹³. Jefferies, on the other hand, meets paraphyly head on. Much of the discussion¹ about *Barrandeocarpus* concerns how this fossil form can be incorporated in a scheme embracing both paraphyly and transformation series.

Jefferies redefines a group of primitive carpoids called cornutes as the 'stem group' of chordates^{3,16}, through which runs the 'stem lineage' to more derived chordates in the 'crown group' (Fig. 2). Each evolutionary novelty along the stem lineage¹⁶ forms the upper or lower bound of a discrete segment of the stem lineage, called a plesion. Stem groups and plesions are paraphyletic in that they cannot be defined by unique sets of derived characters that exclude crown groups. This is because the character that defines the lower bound of a stem group or plesion is also present, at least primitively, in more 'crownward' forms. Jefferies' use of the plesion concept moves it a considerable distance from its original definition (Fig. 2)¹⁷. His addition of concepts such as the stem lineage has turned the (monophyletic) plesion into a (paraphyletic) lineage segment, an idea he has worked out most extensively in the systematic placement of *Barrandeocarpus*¹.

Jefferies defines a relatively derived

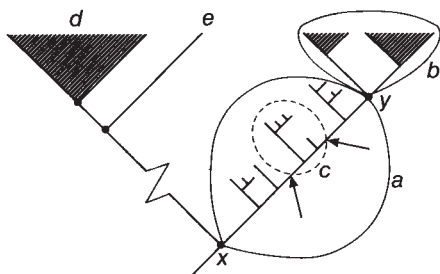


FIG. 2 Some cladistic terminology explained. The right hand side of the diagram shows a stem group *a*, (such as the cornutes), leading to a crown group *b* (such as the mitrates); *c* is a plesion (see ref. 1) defined by evolutionary novelties (heavy arrows) along the stem lineage (between *x* and *y*). The stem group and plesion are clearly paraphyletic. The left-hand side of the diagram illustrates the original plesion concept¹⁷. The fossil taxon *e* is incorporated in a character analysis built using data from living forms only to lie as a monophyletic sister group of *d*, which may contain extinct forms, living forms, or both; *e* is referred to as a plesion, but note that it is a monophyletic group used to express character distributions only, and not a paraphyletic group such as *c* which essentially summarizes a grade of organization.

Rock Festival at British Museum

THIS sample of aragonite from Cumberland features in a new exhibition at the Geological Museum in Cromwell Road, London SW7, now part of the British Museum (Natural History). The 'Rock Festival' features the world of minerals in nature and art and runs until 15 January 1990. Originally developed by the city of Strasbourg as part of its bimillennial celebrations, the exhibition also highlights many of the museum's own specimens. □



group of carpoids, called mitrates, as crown-group chordates which retain calcite skeletons. He suggests that there are three separate stem groups within the mitrates, leading, respectively, to acranians (such as the amphioxus *Branchiostoma*), the tunicates and the vertebrates. Every known mitrate can be assigned to one of these stem groups³. *Barrandeocarpus* is the member of a plesion in the vertebrate stem group within mitrates, but is somewhat removed from the actual vertebrate stem lineage. Defining its precise position has led to a new method of subdividing plesions¹.

Jefferies' theory is by no means generally accepted¹, and the loss of the calcite skeleton is a particular stumbling block. Jefferies claims that calcite resorption can be seen in some mitrates such as *Mitrocybella*, but even if calcite had been lost in this way, many questions about the nature of the calcichordate integument remain unanswered. The only living animals with a calcichordate-type skeleton are echinoderms, and their integuments are fundamentally different in structure and function from those of chordates. Furthermore, the calcichordate fossil record seems to be at variance with the phylogeny reconstructed by Jefferies³. Calcichordates are known from Lower Palaeozoic rocks throughout the world, but almost all known examples postdate the earliest records of true, fish-like vertebrates. So if the calcichordate theory is correct, many examples remain to be discovered in Cambrian and Precambrian rocks. (Indeed, new species of calcichordate are still being found in Wales in the United Kingdom, where Lower Palaeozoic rocks are well-charted¹⁸.) Problems of completeness in the fossil record are best solved by digging rather than theorizing — but making the calcichordate theory fit the harsh realities of the fossil record may

turn out to be its biggest difficulty.

In the meantime, the fossil species Jefferies uses to build his theory³ can be considered as abstract and timeless types rather than as real species, between which there are not evolutionary relationships but only affinities. Pre-evolutionary biologists (such as Linnaeus) classified organisms fairly well without invoking evolutionary change. Cladists accept that evolution happened, but prefer to ignore it as an explanatory process until its pattern has been worked out.

Nevertheless, others (including some of Jefferies' critics) think that the calcichordate theory — as a theory — is the best so far to explain chordate and vertebrate origins, in that it is the most self-consistent. For all its problems, and whether one believes it or not, the organization of an otherwise bewildering array of meticulously well-defined characters according to cladistic principles makes the calcichordate theory a hard act to follow. □

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Heavy photons light up hot nuclei

Philip J. Siemens

By colliding atomic nuclei at relativistic velocities, physicists heat and compress matter to temperatures and densities not seen since the first millisecond of the Big Bang. In the 10 August issue of *Physics Letters B*, Roche *et al.*¹ report new observations of rare electromagnetic signals that promise to illuminate the properties of hot, dense matter made in nuclear collisions. The electron-positron (e^+e^-) pairs they measure carry information about the electric currents in the matter. These data are especially valuable because the pairs are among the few signals able to penetrate the hot, dense matter. The pairs are the material remnants of massive photons, quanta of radiation that exist only ephemerally in the neighbourhood of the currents that produce their electromagnetic fields. The experiments seem to confirm theoretical predictions by Gale and Kapusta² and others³ that the currents from charged mesons, principal agents of the nuclear force, would be seen as bumps in the spectrum of photon masses. Refinements of these measurements could provide unique insight into how mesons behave in the hot, dense matter, which is the key issue unresolved by previous observations of nuclear collisions.

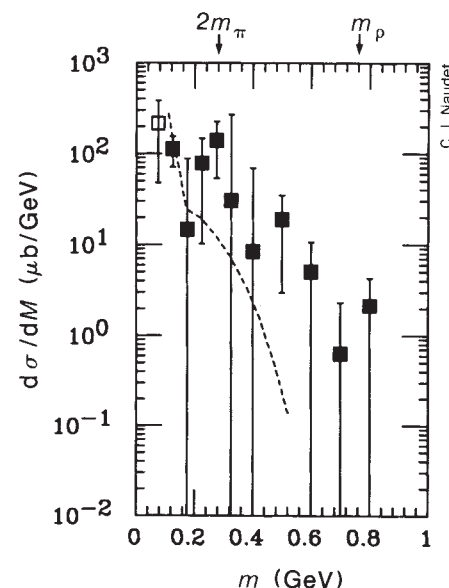
The ability of the e^+e^- pairs to penetrate the hot, dense matter that creates them offers hope of a breakthrough in the huge programme of experiments in which hot, dense matter has been made and studied in the Lawrence Berkeley Laboratory since 1975. A complex of linked accelerators provides beams of nuclei moving as fast as 95 per cent of the speed of light. When these nuclei collide with nuclei in stationary targets, the force of the collision compresses them to a fraction of their original volume, producing matter densities of about 10^{15} g cm⁻³. In the collision, much of the energy initially imparted by the accelerator is converted

to heat, leading to temperatures of about 10^{12} K (for comparison, the core of a type II supernova reaches comparable densities but at much lower temperatures). Unfortunately, this hot, dense matter explodes, so that within 10^{-22} seconds its density has dropped below that of the original nuclei from which it was formed, though its temperature is still around 5×10^{11} K. Until now, observations have been limited to the dilute, cool products of the explosion. Although the properties of the hot, dense matter determine the results of the explosion, the necessary theoretical analysis is so indirect that large uncertainties remain in the deduced quantities: compression energy, heat capacity, collision and reaction rates, and the potential energies of particles in the matter all remain uncertain within a factor of two.

The electrons and positrons observed by Roche *et al.*¹, like their parent photons, remain undisturbed by the violent rearrangement of the matter during the explosion, because they are unaffected by the strong nuclear forces. This advantage is countered by an experimental disadvantage: because the forces that create the pairs are so small, the average time needed to create a pair greatly exceeds the duration of the explosion, and hundreds of explosions must be monitored before an interesting pair is created. The resulting low rates place rigorous demands on experimental technique. Nevertheless, an impressive effort has now led to results for nuclear collisions that seem to contain new information about the properties of the hot, dense matter. These results follow close on the heels of a report⁴ in *Physical Review Letters* demonstrating the method for collisions of protons on beryllium targets. The importance of the new technique is highlighted by theoretical arguments that certain characteristics of the pairs should be especially sensitive to

HEAVY LIGHT Because e^+e^- pairs experience only the electromagnetic interaction, they have to be produced by way of photons, the quanta of the electromagnetic field. An ordinary photon, which propagates at the speed of light c , has no mass; a massive photon, possible only because of the uncertainty principle, can neither exist for long nor propagate far. Every photon, massive or light, is created by an electromagnetic current which provides the photon's momentum and energy. Because momentum p and energy E are conserved, each pair has the same p and E as the photon which produced it; the photon, in turn, receives that momentum and energy from the sources of the current. According to quantum mechanics, the momentum of a particle produced in a localized region of size Δx is indeterminate by an amount $\Delta p = \hbar/\Delta x$; similarly, the energy of a particle existing for a time Δt is indeterminate by $\Delta E = \hbar/\Delta t$ ($\hbar$ is Planck's constant). Thus an electromagnetic field existing in a limited region for a limited time has to be described by quanta — photons — whose energies and momenta are not related by the usual $E = pc$. Although such a photon, with a non-zero mass $m = \sqrt{E^2/c^4 - p^2/c^2}$, exists only ephemerally, that brief appearance gives it the possibility of creating an e^+e^- pair for which both the electron and positron fulfil the required relations between momentum and energy so that they can continue to exist indefinitely.

P.J.S.



The invariant-mass spectrum for the reaction of a beam of Ca nuclei at 0.95c on a Ca target. Solid squares: experimental measurements; open square: tentative analysis; dashed line: contribution due to single-pion decay.

some of the most interesting and controversial properties of the hot, dense matter.

Because momentum, p , and energy, E , are conserved, the p and E of the e^+e^- pair must come from the currents that created them. The pairs may be classified by their invariant mass $m = \sqrt{E^2/c^4 - p^2/c^2}$ which coincides with the mass of the photon that mediates their creation (see box). Pairs with small m are quite numerous, arising from 'bremsstrahlung' radiation due to the acceleration of colliding protons and from an infrequent decay mode of neutral π mesons (pions); the number of pairs from these sources decreases rapidly with m . Pairs with large m are very rare, originating in the annihilation of quark-antiquark pairs. The decay of the ρ meson produces pairs with that meson's mass, $m_\rho = 770 \text{ MeV } c^{-2}$.

In addition to these mundane sources of e^+e^- pairs, Roche *et al.* observe (see figure) many pairs with m less than m_ρ but greater than twice the pion mass m_π . Such pairs can be produced by the annihilation of charged pions $\pi^+\pi^- \rightarrow e^+e^-$. Pairs from pion annihilation would be especially interesting for two reasons: the pion is the main agent of the attractive force that binds nuclei; and the creation of pions is thought to be responsible for much of the heat capacity of nuclear matter. Not surprisingly, pions are strongly influenced by the presence of nuclear matter. The nature and extent of this influence is the most difficult point left unsettled by earlier experiments on colliding nuclei. Because the pions are so crucial to the properties of the matter, the controversy about their behaviour results in uncertainties in the deduced values of all the properties of hot, dense matter.

In 1987 Gale and Kapusta² pointed out

that the influence of nuclear matter on pions should be seen directly in the spectrum of masses of $e^+ - e^-$ pairs from their annihilation. In the absence of matter, the minimum mass of the pair would be $2m_\pi = 279$ MeV. To a first approximation, one might expect hardly any pairs to be created with this minimum mass, both because the masses of most pairs would be increased by the kinetic energy of the pions and because the electromagnetic current, which is proportional to the velocity of the pions, vanishes when they have no kinetic energy. Thus the spectrum of masses would show⁵ a peak well above $2m_\pi$ followed by another at m_π .

However, the energy of pions in nuclear matter is reduced by an attractive potential energy so that, as Gale and Kapusta argued, the position and shape of the two-pion peak in the $e^+ - e^-$ mass spectrum could show the magnitude of the interaction energy. The attraction increases so rapidly with the pions' momentum that the kinetic energy of a pion in the middle of a large nucleus is approximately cancelled by the potential energy⁶ except for those whose momentum p is several times $m_\pi c$. As a result, pions with a wide range of momenta have about the same energy $m_\pi c^2$ in nuclear matter; any two such pions could annihilate to give an $e^+ - e^-$ pair with a mass near $2m_\pi$ (or even less because of the pair's momentum). In dense matter, the pions' attraction could be greater, leading to many pairs of even lower mass.

The figure shows that the spectrum of $e^+ - e^-$ masses measured by Roche *et al.* indeed exhibits a peak near $2m_\pi$, although the statistical uncertainties of the small data sample preclude a precise determination of its location. In the reaction of protons on Be at 4.9 and 2.1 GeV, similar peaks were observed⁴ with better accuracy; after accounting for the low-mass pairs from decay of single pions, those peaks appear to be very close to $2m_\pi$ as expected for nuclear matter at normal density. The interpretation that these pairs arise from two-pion annihilation is confirmed by the observation⁴ that a 1-GeV proton on Be produces only pairs consistent with the single-pion background. This is as expected because 1-GeV protons seldom produce two pions.

Encouraged by their success, Roche *et al.* are pressing ahead with their experimental programme. Before conducting further measurements on nuclear collisions, they are preparing an experiment to study carefully the production of $e^+ - e^-$ pairs in collisions of protons with hydrogen targets to enable an accurate evaluation of how many low-mass pairs are due to bremsstrahlung. Because the bremsstrahlung rate must fall smoothly with mass, it cannot be responsible for the observed peak in the mass spectrum; however, a detailed analysis of the shape and position of the pion-annihilation peak

requires subtracting the more pedestrian processes (the single-pion decay is well in hand). After these studies, Roche *et al.* hope to return to the application of their technique to nuclear collisions, both in Berkeley and at the higher-energy beams now becoming available in Brookhaven.

Theorists are also scurrying to extend their models to interpret these new measurements, which may contain the clue to the riddle of hot, dense matter. □

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CONSERVATION

Desert rhinos dehorned

N. Leader-Williams

THE dehorning of rhinos has finally been attempted in a brave experiment aimed at thwarting poachers¹. Africa's black rhinos *Diceros bicornis* have dwindled at an unprecedented rate, from an estimated 65,000 in 1970 to fewer than 4,000 now, chiefly because of international demand for rhino horn. During the past decade conservationists' attempts to halt this decline using both a total trade ban on horn, enforced through the Convention on Trade in Endangered Species, and underfunded field-based projects to protect large populations *in situ*, have failed miserably^{2,3}. The dehorning experiment was conducted in Damaraland, Namibia, where a small population of black rhinos uniquely adapted to desert life had once again become threatened when 16 of them were killed this year^{1,4}.

The experiment will cause controversy among conservationists because, although dehorning has been discussed as a measure to prevent poaching since the 1950s, until now it has been discarded in most areas of Africa for several reasons⁵. First, the cost of dehorning several thousand rhinos over tens of thousands of square kilometers would be extremely expensive. Second, the two African species, the black and the white (*Ceratotherium simum*) rhino, use their horns in sparring⁶ and to defend

calves against predators such as lions and spotted hyenas (see figure). Hence, hornless rhinos may be unable to maintain their social status or to rear their calves successfully. As important, most black rhinos live in thick bush, and a poacher sighting only a part silhouette could shoot before finding his quarry is hornless⁵.

To stand the maximum chance of success, therefore, dehorning should be carried out in a small and discrete population of rhinos living in an open area where there are no natural predators. These conditions are met by the habitat and rhino population of Damaraland, where the principal factors to be considered are the effect of dehorning on social status and the risks of injury in fights with horned rivals. The other major advantage of Damaraland is that the successful conservation measures that brought the population of desert rhinos up from a heavily exploited population of around 40 in 1980/81 to 100 in 1988 involved local tribesmen who act as auxiliary game guards⁴. Their role in informing potential poachers that rhinos in the area are now dehorned will be vital.

If successful in Damaraland, dehorning may become more widespread. Plans have already been made to dehorn a group of threatened white rhinos in Zimbabwe⁷.

Even though previously rejected as a conservation method for the once large populations of black rhinos living in wooded areas, circumstances may have changed sufficiently for dehorning to be reconsidered in East and Central Africa. Remnant black rhinos in Kenya have been moved during the past few years into small fenced sanctuaries, a strategy which has so far been successful in stabilizing numbers⁵. As a further precau-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

A mother rhino defends her bloodied calf against a hyena. Will dehorning increase predation risk? (Photograph courtesy of Dr Hans Kruuk, Banchory Research Station, Kincardineshire, UK.)

tion, however, all the enclosed rhinos could be dehorned, predators of rhino calves removed from the sanctuaries, and information and education campaigns mounted to inform poachers of the dehorning so that reprisal killings⁸ are avoided. Tourists, too, would need to accept that hornless rhinos are better than no rhinos.

One other problem remains to be solved. The horns of the Damaraland rhinos have only been sawn off and filed down¹, and so will regrow within two to three years. If initial dehorning experiments prove successful and rhinos are not to be exposed to the risks of repeated immobilization, cauterization of the horn bases should be experimented with. An analogous operation, albeit with horns of

a somewhat different structure, is carried out routinely to poll horned breeds of cows and is permanent⁹. Conservationists can only hope that Namibia's courageous move will play a constructive role in a final stand to save Africa's rhinos over the next decade.

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GEOLOGY

Fluid flow through fault zones

Andrew M. McCaig

NEW work in Virginia¹ emphasizes the importance of fault zones as major conduits for fluid flow through the middle crust, and may provide a useful new tool for gold exploration. Gates and Gundersen¹ have found significantly enhanced concentrations of radon gas in soils overlying the Brookneal fault zone in West Virginia, relating this to uranium concentrations of 7–8 parts per million (p.p.m.) contrasted with the 1–2 p.p.m. found in nearby undeformed granite. There is also a rough correlation between the shear strain in the zone and the uranium and thorium content, suggesting a progressive increase in uranium enrichment with deformation.

It has been known for many years that mylonitic rocks in ductile shear zones are frequently very different in composition to the rocks they cut^{2–5}. These chemical changes are generally ascribed to the passage of large volumes of hydrous fluid through the zones. Shear zones are by definition much more strongly deformed than surrounding rocks and are typically

produced when volumes of rock metamorphosed or intruded at high temperatures are reworked under lower-temperature conditions. There is almost certainly a complex feedback mechanism between fluid flow, chemical alteration and strain concentration⁵, but it is very hard to separate cause from effect. Nevertheless, it is clear that the only permeable pathways through large volumes of the continental crust must be shear zones, and that in many cases these will be effective fluid conduits only during active deformation.

It is interesting to speculate on the source of fluid and the driving force for fluid movement in shear zones. In the Appalachians, large blocks of highly metamorphosed basement rock such as the Blue Ridge and Piedmont belts (see figure) have been thrust for tens or even hundreds of kilometres over relatively unmetamorphosed sediments⁶. It is possible that the fluid moving through the Brookneal and similar zones was squeezed out from such sediments owing to the enormous weight of thrust

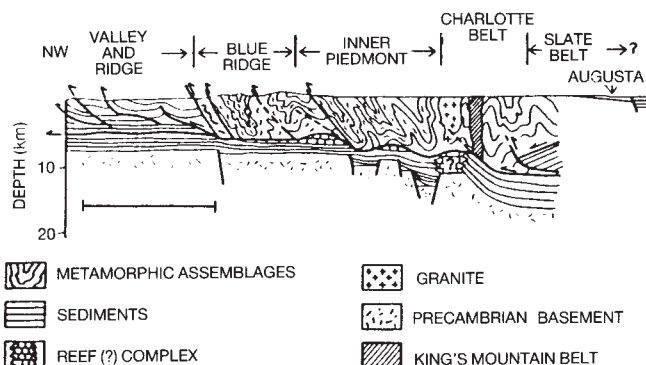
sheets above them⁴. But the Brookneal Zone shows mainly strike-slip motion and was probably active before the main Alleghenian collisional event⁷, which placed the Piedmont Belt over continental margin sediments. In these circumstances, fluid may have entered the shear zone as a result of seismic pumping⁸, either directly or via an underlying decollement⁹. Seismic

pumping occurs because of the development of microcracks in highly stressed rocks immediately before earthquake rupture. This increase in pore volume (dilatancy) leads to pressure differentials and sucking of fluid into the stressed rock. When rupture occurs, the microcracks close up, forcing out fluid and leading to greatly increased spring discharges⁸.

Fluid movement through fault and shear zones is an important subject of study for both environmental and economic reasons. As pointed out by Gates and Gundersen¹, radon gas is a major and insidious environmental health problem in any area where uranium is concentrated in underlying rocks. Clearly, shear zones should be included in the list of potentially hazardous sites. Equally important is the question of waste disposal; there is hardly any area of crust which does not contain either ancient or currently active fault zones. It is vital to assess the role of seismic pumping if waste is to be buried in seismically active areas such as California, and the long-term permeability of fault and shear zones must be established even in stable regions. A matter of current concern in waste disposal is the presence of corrosive hypersaline brines as groundwaters in crystalline rocks¹⁰. One possible source for such groundwaters is leaching of ancient fluid inclusions¹¹. Recent work in Leeds (S. Tempest, personal communication) shows that fluid inclusions from altered shear zones can contain up to 40 per cent by weight equivalent of NaCl. The possibility that shear zones may contain anomalous concentrations of ancient, corrosive fluids has implications for many engineering projects.

Finally, fault and shear zones are prime targets for gold exploration in many parts of the world. Mineralization is commonly associated with specific geometrical features such as bends and intersections of zones¹². Often the location and geometry of shear zones are concealed by superficial deposits so that soil sampling for radon may prove a powerful exploration tool. □

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Cross-section through the southern Appalachians based on a COCORP seismic reflection profile. The Piedmont belt, containing the Brookneal shear zone studied by Gates and Gundersen¹, is interpreted to overlie unmetamorphosed continental margin sediments. (From ref. 6.).

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Detergent ringing true as a model for membranes

J. Barber

It is a frustrating business trying to understand how specific lipid species seem to optimize the functional properties of proteins that are embedded in the lipid bilayers that constitute most biological membranes. The problem is that membrane proteins are hydrophobic and therefore difficult to isolate and purify without using detergents to disrupt the membrane. Consequently, detergent molecules replace the lipid, and the best one can hope for is that information — for example, from high resolution X-ray crystallography — on how the detergent is organized in relationship to the protein will be of some relevance to how the natural lipids are organized. Even then, the approach is hampered by the fact that detergent molecules will often not form ordered arrays because of thermal motion, particularly in their hydrocarbon tails. On page 659 of this issue¹, Roth *et al.* report an alternative approach, which makes use of neutron scattering.

Roth *et al.* have studied highly-ordered crystals of the reaction centre of the photosynthetic bacterium *Rhodospseudomonas viridis*, isolated with the detergent lauryl dimethyl amine oxide (LDAO). X-ray defraction of these crystals has shown that the reaction centre has a hydrophobic core consisting of eleven membrane-spanning α -helices, five each from the L- and M-subunits of the protein and one from the H-subunit². To be able to investigate the arrangement of detergent in the crystals, Roth *et al.* use the trick of varying the H₂O/D₂O content of the crystals, recording variations of the phase of the structure factor as a fraction of contrast.

Unlike X-ray crystallography, this approach gives the low-resolution data needed to identify the domains formed by the detergent molecules in the crystals. Under optimal conditions, the authors observe the detergent as micellar rings around the transmembrane α -helices of all three subunits. The thickness of the detergent rings along the α -helices is 25–30 Å, about twice the length of an LDAO molecule. Therefore, it seems that LDAO arranges itself around the hydrophobic core of the protein complex in the way depicted in text books for natural membrane lipids — as a bilayer.

Recently, Yeates *et al.*³ have conducted minimum energy calculations for the possible organization within a lipid bilayer of the reaction centre of *R. sphaeroides*, which has an atomic-resolution structure⁴ that is remarkably similar to that of the *R. viridis* reaction centre. Their analysis, however, predicts a thicker hydrophobic

region for lipid-protein interactions of about 40–45 Å. The most likely explanation for this difference is that the bacterial reaction centre does not exist as a separate entity within the membrane but normally forms part of a much larger complex involving the proteins that bind the light-harvesting bacteriochlorophylls⁵.

The neutron-scattering experiments of Roth *et al.* have also shown how the detergent domains interconnect throughout the crystal lattice. The results quite clearly show that LDAO not only interacts stereo-specifically with the hydrophobic region of the reaction centre but also creates optimal spacing for direct interactions to occur between the hydrophilic

regions of adjacent proteins. Apparently, it is the interplay of these two factors that is vital for obtaining highly ordered lattices.

The message, therefore, is that there is no 'magic' detergent for crystallizing membrane proteins and each system must be explored independently. Moreover, it is likely that a similar message will apply to the packaging and organization of membrane proteins in their natural environment and that this underlies why nature has not been able to evolve a 'standard' lipid species for all systems. □

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FLUID MECHANICS

Oil slicks still the waves

John C. Scott

THE effect of oil in calming waves is not only one of the oldest phenomena in the scientific calendar¹, it is one which still excites much interest. A recent paper by Alpers and Hühnerfuss² provides a timely summary of recent progress in understanding the calming effect of oil films on the sea surface. It also reports important progress in establishing the link between the processes of wind-energy input, wave-energy re-distribution and energy removal.

Contrary to popular belief not all slicks come from spilt crude oil. The ocean's natural productivity produces much harmless wave-damping material, and it is vital to distinguish the two causes. Concern over pollution, interest in the climatic influence of the sea surface, and the advent of powerful methods of observing the sea from space all ensure continuation of our interest in wave-damping effects.

The basic mechanism of wave damping was given by Horace Lamb, in the 1895 edition of his *Hydrodynamics*³, although the finer points still attract attention (and some controversy). Surface-active chemicals reduce the surface tension of water and the effect increases with the surface concentration of the chemical; when a wave stretches and compresses the surface it increases and decreases the surface tension, causing tangential boundary stresses which always oppose the wave motion. The film gives the surface a 'dilatational elasticity'; in the language of linear hydrodynamics the surface boundary condition changes from a 'free' surface to one which supports tangential stress, and a significant viscous boundary

layer results. Note that the lost wave energy passes (as heat) into the water: it does not directly heat the film.

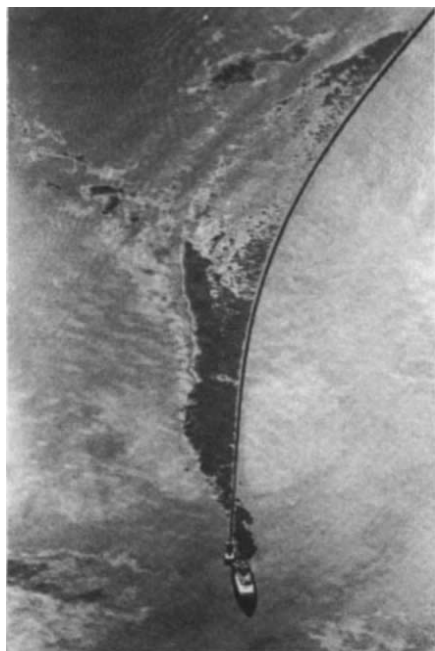
Inextensibility is the upper limit of elasticity, although real films never reach this. One of the most interesting effects of films is that the maximum damping can occur at quite small values of elasticity. Perhaps most remarkable is that the most effective films come from insoluble materials spread as single molecular layers; the effect seems quite disproportionate in this light.

A film usually gives a sharp damping peak, about twice that predicted for the inextensible case, and peaks appear in the variation of damping with wave frequency as well as with film elasticity. Alpers and Hühnerfuss prefer to consider these peaks as 'resonant' energy exchange between two wave modes — one the normal transverse surface waves and the other a highly damped longitudinal wave where motion is mainly to and fro at the horizontal surface. These latter waves are often called Marangoni waves, honouring the contribution to surface-film studies made by the nineteenth century Italian physicist. Although it is often found intuitively useful for considering the damping peaks, this division of the hydrodynamic solutions is not necessary (N. Thomas, personal communication). The conclusions reached by Alpers and Hühnerfuss are, however, not affected by this.

One further interesting influence on wave damping is that the film need not be purely elastic. Viscoelasticity can arise from molecular factors in the film, and

from film solubility, which allows time-dependent diffusive interchange between surface and bulk, and acts to reduce the surface tension gradients.

These considerations relate to all waves propagating on water. However, the wind-blown ocean is clearly more complicated. The primary damping effects of surface films are usually found with wavelengths less than about 100 mm, whereas wind-tunnel and sea experiments on wind



The *Exxon Valdez* lies grounded in the Prince William Sound, off Alaska. The thick slicks formed by crude oil are less surface active than biogenic slicks, and may be distinguished by analytic techniques.

waves all show very large effects on waves several metres long².

The novel step taken by Alpers and Huhnerfuss is to bring together wave-damping theory with recent results on energy exchange within the surface-wave spectrum, in particular nonlinear wave-wave interactions. The authors draw on recent results by Hasselmann and Hasselmann⁴ and Phillips⁵ concerning the distribution of energy within the spectrum and the rate of energy input from the wind.

The mechanism proposed follows the existence of the damping peak in the frequency variation, often near 5 Hz. Wave spectra on film-covered surfaces are duly found to have a pronounced dip at the corresponding wavelength (50–100 mm). It is proposed that the effect at much longer wavelengths comes from nonlinear wave interactions acting to re-establish the normal equilibrium spectrum, draining energy into the region of the minimum, and away from the longer waves. The authors present approximate calculations which suggest that such a mechanism would indeed explain the observations. They also give preliminary computations in support of the conclusion, although

truly quantitative results were not possible because of computer limitations.

The linking of two such complex theoretical areas as film damping and wave-wave interactions is not straightforward, and several marginal assumptions have to be made, such as that the nonlinear process itself is not primarily affected by the film, and that other energy 'sinks' such as wave breaking are unaffected. Nevertheless, the results are encouraging when the calculations are compared with laboratory and sea data. The latter include both wave-amplitude and radar backscatter measurements, these being crucially important for the detectability of slicks by real-aperture or synthetic-aperture radars.

Experience of some form of slicks is common. Sun-tan oil can be seen to spread from summer bathers, and on very hot days intricate near-shore patterns of slicks can be seen. These are almost certainly from natural underwater biological activity — fish or plant life — rather than pollution. Even many hundreds of miles from civilization, a calm sunny day will give a 'glassy' sea. Such slicks are frequently unfairly attributed to polluters, and it is therefore vitally important to discriminate slick causes.

Fortunately, major pollutants such as crude oil (see figure), fuel oils and lubricants are usually quite different from biogenic slick materials. They are much less surface active, and they therefore tend to form thicker layers, spreading less. This leads to differences in radar backscatter, and Alpers and Huhnerfuss cite a paper in press on just this topic. There can be even more significant differences in the lidar, thermal infrared and fluorescent ultraviolet signatures of slicks, which could be detected by a multi-sensor approach, such as that adopted in the United Kingdom by the Warren Spring Laboratory.

Slicks also play an important role in ocean research. Natural slicks have a tendency to become aligned with regions of strong shear, and a remote sensing technique such as imaging radar⁶ or sunglint⁷ can thus provide valuable oceanographic information. The increasing availability of space-borne sensors, such as the radar-imaging satellite ERS-1, will ensure interest in surface films for many years to come. □

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Flying dust

MODERN detergents have done much to ease the never-ending problem of keeping things clean. A detergent molecule has an oleophilic end which binds to a greasy dirt particle, and a hydrophilic end which remains solvated by the wash water. So the adhering molecules 'couple' the dirt particles to the water. They lift off the surface to be cleaned, and come into liquid suspension.

Daedalus now points out that exactly the same mechanism could apply in air. Dust is hard to blow off a surface because its particles are poorly coupled to the air. If they could be covered by adsorbed molecules which interacted strongly with the air, they would lift off much more readily. So Daedalus is inventing a gaseous detergent.

He is synthesizing molecules whose hydrocarbon 'head' readily condenses onto greasy dirt. Their 'tail' is some long, light, flexible chain presenting a big collision cross-section to air molecules, probably a fluorocarbon (hydrocarbon chains, sadly, are oleophilic). Many fluorocarbons are extremely volatile for their molecular weight, and should have a powerful air-lift action on dirt particles.

But the new detergents will not be a gas. For by analogy with water-based detergents, it should work best at concentrations around 0.1 per cent, or a partial pressure of 0.001 atmospheres. And since it must condense out of the air and be adsorbed hygroscopically on the dirt at this concentration, this must be its saturated-vapour pressure at room temperature: implying a boiling-point around 180 °C. So 'Aerosolve' (as it will be called) will have quite a high molecular weight, with room for much chemical subtlety.

Its first mass application will be as a vacuum-cleaning aid, wafted ahead of the machine by an evaporator on its cleaning head. The stickiest dust will lose its grip on the carpet and be effortlessly sucked up. More radically, a room permanently saturated with Aerosolve would never get dirty in the first place, for the dust would stay harmlessly in air-suspension. Just hang a wick or paper strip saturated with Aerosolve in the room, and your cleaning problems are solved. The gradual insidious dirtying of all surfaces, which only becomes apparent when you rub a finger over them, or take a picture down and find a clean patch behind it, would simply not happen. The slow fouling of air-cooled laboratory equipment, which makes the servicing of old computers, and so on, such a grimy experience, could similarly be prevented.

Even better, Aerosolve vapour in the air should prevent dust and bacteria being deposited in the lungs as well. The benevolent detergent-vapour could thus help to prevent pneumonia, asbestosis, and some of the nasty diseases that afflict smokers.

David Jones

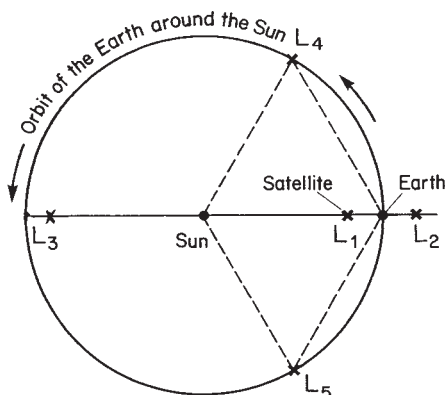
Mirrors to halt global warming?

SIR—It seems very likely that little will be done in the future to reduce the emission of greenhouse gases. If global warming is confirmed, a remote but feasible possibility to reverse the temperature increase is by changing artificially the solar radiation incident on the Earth.

The radiation balance of the Earth is controlled by the Stefan–Boltzmann law:

$$\epsilon\sigma T^4 = (1-A)S_0/4 \quad (1)$$

where the left-hand side is the infrared black-body radiation from the Earth and the right-hand side represents the mean solar (visible) radiation on the Earth. Here A ($=0.3$) is the albedo of the Earth, S_0 ($=1.372 \text{ kW m}^{-2}$) is the solar constant near the Earth, σ ($=5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$) is the Stefan–Boltzmann constant, and ϵ ($=0.62$) is the emissivity of the Earth with its atmosphere. Equation (1) gives $T = 288 \text{ K}$ as the mean temperature on the



The five Lagrange points L_1 – L_5 for the three-body system (Sun, Earth and satellite). In our case L_1 is chosen as the position for the mirror-bearing satellite.

Earth's surface.

To compensate for a temperature increase of ΔT originating from the greenhouse effect, we have to reduce the relative radiation ΔS_0 by

$$\frac{\Delta S_0}{S_0} = 4 \frac{\Delta T}{T} \quad (2)$$

For example, to compensate for a temperature increase of 2.5 K , the solar radiation must be reduced by about 3.5% . The task could be done by satellites bearing large lightweight mirrors. However, satellites in 'normal' orbits do not cast their shadows permanently on the Earth.

This difficulty may be overcome, and the 3.5% reduction achieved, with a minimum mirror area of $4.5 \times 10^6 \text{ km}^2$, by positioning a satellite in such a way that it will always stand between the Sun and the Earth, permanently casting its shadow on the Earth. In this context my attention has been drawn to some particular solutions of the three-body problem, the so-called Lagrange points.

In the plane of the elliptical orbit of a planet around the Sun there are five Lagrange points, L_1 to L_5 (see figure), which are characterized by the fact that if a third body (a satellite, for example) is positioned at one of these points, the geometrical figure between the body, the Sun and the Earth remains constantly self-similar. For our purpose the so-called collinear solutions, based on L_1 , L_2 and L_3 , are the most important, particularly Lagrange point L_1 . If a satellite was positioned at L_1 it would remain on a straight line between the Sun and the Earth at all times and could cast its shadow permanently on the Earth. Thus, this would make possible a reduction of the solar radiation power on the Earth using a minimum of mirrors.

If we assume that the mass of the satellite is negligible relative to the mass of the Earth, then the distance between L_1 and the Earth is $d \approx 1.5 \times 10^6 \text{ km}$, or about four times the distance between the Moon and the Earth (K. Stumpff *Himmelsmechanik* Vol. II, 104; VEB, Berlin, 1965). The satellite would have an infinite synodical period and its sidereal period would be the same as the Earth's (that is, one year).

How much energy will be required to bring mirrors of the necessary size to the point L_1 ? If we assume that they consist of aluminium of mass 10 g m^{-2} , at least 45 million tonnes of material will have to be

brought to L_1 . We estimate that the energy required to do this is equivalent to the output of 30 nuclear power stations producing 1 GW for 20 years. A very crude estimate of the cost of this venture gives, over 20 years, a figure of 6% of the present annual gross national product of the world per year, which is roughly equivalent to its total annual military expenditure.

It should be noted that the Lagrange position L_1 is an unstable position: there is no negative feedback mechanism to hold the satellite in position should it drift away for any reason, for example because of the light pressure of the Sun on the mirror. Furthermore, the Lagrange solutions are strictly valid only for a pure three-body problem. Although the attractive force of the moon on L_1 is at most only 2% of that of the Earth, this will have some influence. Thus the satellite would have to be stabilized actively, which requires permanent observation and maintenance. However, the solar light pressure is just strong enough to allow a robust active control of the equilibrium of the mirror.

Of course, there are other important aspects which have not been considered, the most critical of which is whether it is possible to produce reflecting foils with a mass of only 10 g m^{-2} which will be able to withstand mechanical forces, solar winds and so on for a long time.

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Drying, O_2 and mass extinction

SIR—Reports^{1,2} of a drop in the $^{13}\text{C}/^{12}\text{C}$ ratio of the oceans during the late Permian have been interpreted as a result of an input of light carbon to the oceans from the oxidative weathering of pre-existing organic matter, either on the ocean floor² or on the continents¹. Increased weathering on the continents is attributed to the lowering of sea level and the exposure of additional organic-containing sediments to the atmosphere at that time¹. Weathering of organic matter results in the uptake of O_2 from the atmosphere, and a lowering of atmospheric oxygen might have contributed to the Permo/Triassic extinction², the most extensive mass extinction to occur over all of Phanerozoic time³.

Although increased organic oxidation during the latest Permian might have been important, another factor which should have contributed to a more gradual lowering of atmospheric oxygen during the mid-to-late Permian, is the general trend towards greater continental aridity, resulting in a lowering of worldwide organic matter burial. Burial of organic matter in sediments represents net photosynthesis and net oxygen production; thus, any

large drop in worldwide organic burial should result in a drop in atmospheric O_2 .

Because of the rise of vascular land plants and the deposition of their remains in terrestrial coal swamps and, by means of river transport, in the oceans, there was a considerable increase of worldwide organic matter deposition during the Carboniferous and Permian periods compared with earlier times. This conclusion is based on independent modelling of the carbon and sulphur isotope composition of marine sediments^{4,5} and of the relative abundance of different types of sedimentary rocks⁷. (Isotope changes resulting from burial of organic carbon on land are sensed by the oceans by means of carbon transport to the sea by rivers and the atmosphere.)

One can combine organic burial rates with various assumptions about organic weathering to calculate changes in the level of atmospheric oxygen. The figure shows an example for the late Palaeozoic/early Mesozoic, based on the rock-abundance model⁷. Note the high sensitivity of atmospheric O_2 concentration to the value assumed for the average total organic carbon concentration in coal-basin sediments. A reasonable average value for the concentration of total organic carbon, present both as coal plus

Trouble in scholarland

David Joravsky

Intellectual Compromise: The Bottom Line. By Michael T. Ghiselin. Paragon House, 90 Fifth Avenue, New York, New York 10011, USA: 1989. Pp. 226. \$24.95.

MICHAEL Ghiselin has bared his soul in a protracted grumble against the world's affronts to virtuous scholars like himself. One's first impulse is to turn away in embarrassed silence, but the author's eminence — he is an outstanding biologist and historian of darwinism — demands serious consideration, and there are in fact some significant ideas within this tedious flow of basic truths that the dominant fools, rascals, hypocrites and liars of the academic world refuse to accept. "Liar" is Ghiselin's favourite accusation; I cannot remember hearing the epithet used so frequently or so loosely by anyone older than twelve. But I will pass over that mannerism and concentrate on the author's reasoning.

Ghiselin calls his book "social criticism", invoking as his models Tocqueville, Taine and Veblen. He confesses that he cannot truly imitate them, since his "intellectual roots are in zoology, not political science". But economics is the key to understanding human society, and economics is "a branch of biology in a broad sense", and natural selection is the *passé-partout* of biology. So an evolutionary taxonomist who has studied some economics is sufficiently equipped to diagnose the ills that afflict — well, maybe not society at large, which appears only briefly in Ghiselin's complaint, but certainly the learned part of it, which shares the common appetite for the trashy commodities that offend the author's soul.

Fraud in science

For example? The US system of distributing research funds and choosing articles for publication, which fosters mediocrity, trash and outright fraud in science. For *particular* example? I could find no actual specimens displayed in this book, which is full of irascible snorts and grim muttering about bad science but is curiously shy of providing illustrative instances. Ghiselin's closest approach is in reporting a test performed by Peters and Cecci. These authors made typed copies of articles in psychological journals, inscribed John Doe names with Upson Downs institutional affiliations, and resubmitted them to the same journals that had published them with known names and respectable affiliations. In only rare instances did editors or reviewers notice the fraud, reviewers nearly always finding substantive or "methodological" reasons to reject. Does this reveal something about psychology, or about all the

scientific disciplines? Ghiselin assumes the latter, without any attempt to prove the point. I kept expecting a polemical exposé of mediocrity and trash in the biological sciences, especially in those areas where Ghiselin complains of trendy overfunding. But I found only smoky grumbling, no fiery illumination.

So Ghiselin's plea for good honest science in place of dishonest trash remains obscure. And his grand vision of biology and economics? Ghiselin is intent on persuading us that the historical approach to all living things is essential to biology, which thus becomes the instructor science for economics and human history. He provides brief reviews of some divisions among evolutionary biologists concerning the application of an historical viewpoint in their field, informing us of his major disagreements with G.G. Simpson, Stephen Gould, and even with the revered Ernst Mayr, who saved evolutionary biology at Harvard from destruction by molecular reductionists. (Mayr grates on Ghiselin by denying that there are laws of evolution in the same sense as there are laws of mechanical motion.) In other universities — my own, for example — the destructive reductionists have triumphed, and I looked eagerly for Ghiselin to dissect their stunted vision of the living world. Instead he tells all too briefly and enigmatically of his sympathetic interest in molecular biology, mentioning the grants he has received to work out its relevance to systematics. I was left wondering what inward division or restraining caution kept him from telling off representatives of the "nonhistorical, experimentalist ideology" that presses evolutionary studies towards extinction.

Puzzlement vanishes when Ghiselin comes to economics and human history. With clichés and caricatures he bounds over "Marxism", "historicism" and "cultural evolution", to reach a grand explanatory scheme that has somehow been overlooked by specialists in the disciplines of history and economics. Natural selection, supplemented by the economic principle of marginal utility, explains all. Reproductive success or failure of the species is the "bottom line", and marginal utility accounts for the human choices that will ultimately determine that success or failure. This simple formula enables Ghiselin to present his personal tastes as biological imperatives of reproductive success for the human species. It exempts him from the economist's entanglement in

complex calculations of possible choices and results, and from the common historian's entrapment in endless interplay between the imagined past and the real past, not to mention the unknown future. Ghiselin is above all that. He offers brief synopses of his preferred solutions to disputed issues in biological evolution, brushes aside disciplined studies of human affairs in favour of global declarations about marginal utility, and is free to put the stamp of science on the intuitive judgements that have been shaped by personal experience of life, mainly academic, in the United States.

Generalities

Such judgements form the bulk of this sermonizing book, a tedious collection of banalities and dubious generalities concerning science and life, enlivened by autobiographical touches here and there — why the author moved from the revolting university setting to a civilized museum, how his book on the economy of nature was misinterpreted, how, "in presenting talks to academic audiences I have been accused of corrupting the youth — a charge that has also been leveled against people like Socrates, Jesus and Machiavelli". Such colourful accents are unfortunately rare: bromidic grey prevails. The reader who fears that I am being unfair can try these specimens: "Science invents theories and tests them by gathering data, and both of these activities are indispensable." "Prizes are one way of rewarding people for good work." "Scientists ought to be recruited from the ranks of the honest." "The leading scholars tend to be associated with the leading universities." "What academia really wants is a product that sells and gets no complaints from the customer."

The last generality is part of Ghiselin's central complaint against the academic world that shaped his mind and came to disgust him. One could as easily argue the opposite — that academia has standards other than customer satisfaction — and Ghiselin intermittently does so. He goes back and forth between the complaint that shoddy goods dominate the market-place of academic thought and the confidence that "a good theory drives its inferior competitors out of the market". Examples can be given to support either side of the argument, depending on one's tastes and preferences. Ghiselin, as I have said, does not provide examples to show what he means by the shoddy goods that dominate the market-place in biological science, but he becomes specific, and occasionally abusive, about philosophers and historians of science. Thus, Feyerabend's rule that "anything goes" in creative science "was just the sort of attitude that was popular with the Telegraph Avenue drug culture of accursed memory". And he calls William Coleman and Garland Allen

spinners of marxist fairy tales for showing how Haeckel's speculations were at odds with the chief trends in biological science.

That last bit of polemics is more revealing than Ghiselin intended. Coleman, who was president of the history of science society until his death from leukaemia last year, was as far as one can imagine from any form of confessional marxism, and equally far from the habit of using 'marxist' as a dismissive epithet rather than an eponym, like 'darwinian', for one of the most important trends in modern thought. As for fairy tales in place of painful reality, one must ask why Ghiselin is so anxious to rescue Haeckel from his reputation as an irresponsible spinner of evolutionary explanations for everything. Coleman and Allen annoyed Ghiselin by showing how comparative embryology was pushed aside by experimental embryology at the turn of the century, with far more serious consequences than the decline of esteem for Haeckel. What Ghiselin angrily calls "nonhistorical, experimentalist ideology" gathered force in departments of biology until the very existence of evolutionary studies was threatened. Ghiselin has reason to feel dismayed, but hardly to jump on the historians who have told what happened, especially not on such scholars as Coleman and Allen. Their histories of biology are as respectful as he is of systematics and evolutionary studies, including comparative embryology. Once again, Ghiselin fails to engage in polemics with biologists who promote molecular reductionism, and vents spleen on individuals who are actually his comrades on fundamental issues.

As an historian of left-wing movements, I see here a depressingly familiar pattern. When the left is in greatest peril, pressed towards extinction through declining numbers, internal squabbling begins to prevail over the criticism of power that is the left's reason for being. Ghiselin will no doubt be startled to find himself identified with the left, if only in the little world of biologists. I intend this as a compliment, and I offer as conclusive evidence his praise for "The Commoditization of Science" by Lewontin and Levins. (See their collection of marxist essays. *The Dialectical Biologist*.) Indeed, I would endorse his praise of that essay, which lays out the main problem that bothers Ghiselin, and does so more effectively.

Let me state the problem as I perceive it. Modern society, whether in its capitalist or its socialist forms, tends to change scientific research into the production of commodities for sale rather than the quest for truthful knowledge, not to speak of wisdom. Different strata of scientists have been affected in different degrees, as Lewontin and Levins effectively point out. (Ghiselin's alternative description of the strata — rascals, fools, hypocrites,

liars and honest souls — is less useful.) Although most US scientists are directly involved in commodity production, working either as hired hands or as bosses in the service of profit rather than truth, a minority cling to the intellectual autonomy of the traditional artisan or the traditional client of royal patrons. But intellectual autonomy tends to be undermined even within that minority by several forces, the most obvious of which is the bureaucratization of patronage, that is, the modern system of grant-getting. It tends to reward those who seek to satisfy a bureaucratic hierarchy, rather than those who seek truth, not to speak of wisdom.

I have deliberately injected troublesome concepts — intellectual autonomy, truth, wisdom — for a life in science is supposed to approach those priceless goals, and the effort to discuss basic problems of science without reference to them confuses rather than clarifies the issues. Nor can the discussion be limited to abstract philosophical analysis. Anxiety about the commoditization of science is part of a pervasive worry in all areas of modern life. Modernity requires and at the same time rebukes the autonomous mind, the self that would truly know how it stands in relation to the world at large.

Round in circles

Lee A. Segel

Free Energy Transduction and Biochemical Cycle Kinetics. By Terrell L. Hill. *Springer-Verlag*: 1989. Pp. 119. Pbk £19.

THERMODYNAMICS is a difficult subject for many scientists, including myself. The difficulty lies in the fact that thorough understanding requires full intuitive appreciation of macroscopic entities (such as entropy and various forms of free energy) and their relationships. Yet these relationships can only be derived in an entirely convincing fashion by probabilistic analysis of underlying kinetic models. The subtlety of the analysis required is attested to by the continuing generation of important new results, even for what seem superficially to be relatively simple situations.

For decades, Terrell Hill has been a major figure in such 'thermostistical' research (although ugly, 'energo-statistical' might be a better neologism, for at least in the biological sciences energy transduction and dissipation often lie at the centre of attention, not temperature changes). The book under review is essentially an abridged, simplified and updated version of his *Free Energy Transduction in Biology* (Academic, 1977), which in turn was primarily a distillation of his own research. The present title is more accurate, for a considerable amount of material on cycle

Variations on that theme appear not only in *The Communist Manifesto*, where Lewontin and Levins found it, but also in Spinoza and Rousseau and Schiller, where Marx found it. A roll-call of famous thinkers who have developed striking versions of it would be impossibly long.

Let me note merely that scientists are quite mistaken if they regard this as a problem only for philosophers or imaginative writers. When Einstein declared that most scientists are imprisoned in the "traditions of the herd" (see Einstein-Born *Briefwechsel* 203 (1969)) by their training in "mechanized and specialized thinking", he was echoing the Nietzschean version of the anxious theme. It is far too complex to be dealt with adequately here, but it needs to be mentioned, for the greatest deficiency of Ghiselin's book is its unawareness of the tradition within which his questing mind is thrashing about. I urge him to read Max Weber's great lecture, "Science as a Vocation", to get a sense of the tensions that moved him to write this book. □

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kinetics is included. All but the simplest biochemical reaction schemes contain cycles (with accompanying constraints imposed by the requirement of microscopic reversibility).

The basic model treated consists of a number of discrete states with assigned constant transition probabilities per unit time between every pair of states, in both directions. The model can be represented by a diagram (or 'graph' in the more modern mathematical sense) consisting of vertices corresponding to the states and arrows corresponding to the transitions allowed. Many important problems arise, such as determining efficient and intuitively satisfying ways to calculate the rate at which a given cycle on the diagram is traversed, or the mean number of cycles before a final absorbing state is attained. These matters are purely probabilistic. Thermodynamics enters when free-energy levels are calculated for the various states, and fluxes are related to forces that are proportional to differences in free energy.

The book is clearly written, with a commendable plan of illustrating concepts by detailed treatment of carefully selected examples. Although no exercises are provided, this slim and interesting volume indeed fulfils its goal "to be a textbook for a class or for self-study". □

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The natural diagnosis

Roy Porter

Forces of Change: Why We Are the Way We Are Now. By Henry Hobhouse *Sidgwick and Jackson: 1989. Pp. 264. £17.95.*

IN HIS best-selling *The Rise and Fall of the Great Powers*, Paul Kennedy offered a historian's reading of 'why we are the way we are now'. The global dynamics of East and West, of First, Second and Third Worlds, depend in the short run upon military strike power, but fundamentally upon political will and industrial strength. The world's future hangs upon super-power rivalry.

In *Forces of Change*, Henry Hobhouse offers a second opinion upon our problems and prospects. His diagnosis diverges radically. Mankind's true destiny, he argues, may have little to do with such strategic rivalries — he barely conceals his contempt for politicians and all the politicians' men. Rather, the true dynamics of history — too often ignored by blinkered historians — are biological and ecological. Spain conquered the Indies not by superior fire- or faith-power, but because it had smallpox on its side. The United States became the world's top dog not because of democracy and freedom, but because of infinite natural resources, above all, fertile land. The 'forces of change' are, at root, biological.

The natural history of man, he contends, results from a triangle of forces: population growth, food output, and disease. Around 1800, the pioneer demographer, Thomas Malthus, argued that progress was an impossibility, because man's propensity to breed would always outrun food supply: famine, pestilence or war would inevitably ensue. Have we at last succeeded in escaping these malthusian 'checks'? That is the question.

Superficially, at least, the answer might seem to be yes. Medicine is disarming disease. By consequence, global population has doubled twice this century and will do so again before 2030. War has barely culled these numbers, and only local, not global, famine has intervened. This is because food yields continue to rise phenomenally where efficient husbandry, science and technology, pesticides and the 'green revolution' have been shrewdly used. Future biotechnological prospects look rosy.

So is Malthus defeated? Hobhouse's answer — in a book by turns angry and witty — is basically no. Certainly, we can multiply population, but on most continents all we do is multiply misery, as peasant subsistence farming disintegrates

and the despairing masses flock to nightmare cities. Malthusian checks themselves may well have the last laugh with AIDS. And, of course, so-called progress is precipitating a new range of checks unknown to Malthus — the greenhouse effect, acid rain and the destruction of the ozone layer. Recognized all too late in the day, environmental pollution is perhaps already irreversible.

So far, so familiar. Hobhouse argues his brief with energy, combining historical vision with an expert grasp of agricultural business; but the basic analysis (condition chronic, possibly terminal) is hardly news. What rescues this diagnosis from the commonplace is its plain-man's trenchant realism, dismissive of pieties, platitudes and pie-in-the-sky.

Almost without exception, planners' attempts to cure by *diktat* the problems of population and hunger have proved counter-productive. From Stalin's Russia to Ethiopia, state socialism has been an eco-disaster. Hobhouse shows no more sympathy for high-minded movements like Greenpeace (sentimental, self-indulgent hot air), or international aid agencies, which palliate today's problems only to worsen tomorrow's. Native ways were wiser than Western do-gooding: better to have infanticide and high perinatal mortality on the savannah than surplus millions rotting in refugee camps. At least such folkways weeded out the 'unfit'.

And here, in this tell-tale term, harking back to social darwinism, we have the clue to Hobhouse's own nostrums. Like all other creatures, humans flourish when their habits 'fit' a particular environmental niche. The neo-Europes, New Zealand above all, have achieved a healthy 'fit' between human demands and natural resources. Japan's success is due more to its super-efficient land husbandry than to technological wizardry. In other words, Hobhouse sets store by a kind of territorial imperative: every population must carve out its own *modus vivendi* with nature, and then protect that survival strategy.

This requires a healthy individualism. Paternalism and egalitarianism are out in Hobhouse's tough-minded neo-darwinism: the idea of free health care at the point of service is a nonsense, he asides. We mistakenly look to leaders for magical answers, he says. Vest (some) faith in science, but none in politicians, for know-all, vote-seeking governments generally turn out to be environmental hazards.

Hobhouse's commitment to nature's



Sweet as honey — a mesolithic rock painting (7,000–4,000 BC) from Cuevas de la Araña, Bicorn, Spain, depicting a woman with a basket gathering wild honey from a hive at the top of a tree. The picture is taken from *Women in Prehistory* by Margaret Ehrenberg, published by the British Museum.

laws, to knowledge and enterprise, and his hatred of humbug has an attractive ring — as, of course, had Victorian social darwinism. But, for all its trumpeted 'realism', it has its own fatal blind-spot. For in the modern world you cannot divorce nature from politics. It is all very well to assert that the real problem of South Africa is not apartheid but the Bantu population explosion and consequent agricultural under-capitalization; but it takes a certain wilful myopia to treat these as unconnected. Trust to enterprise, Hobhouse urges, and you minimize waste. Maybe. But maybe you also end up, as he himself admits, with Thatcher's Britain disgorging vast quantities of untreated sewage and pollutants into the North Sea (and surely that is waste, by anybody's definition, not to say ecologically stupid).

It is disturbing to find a born-again darwinian convinced that enlightened competitive individualism will rectify today's ecological imbalances. For was it not these very 'forces of change' that first created the problems? Yet in these paradoxes lies the genuine stimulus of Hobhouse's spiky and often opinionated analysis. By tilting at conventional wisdoms, he forces us to think hard and deep about our true relationship with our planet. □

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Honesty's the best policy

J. L. Heilbron

Science à la Mode: Physical Fashions and Fictions. By Tony Rothman. Princeton University Press: 1989. Pp.207. \$19.95, £12.50.

IN THE middle of the eighteenth century, the Berlin Academy of Sciences advertised an essay competition on the question, "Is it useful for men to be deceived?". In so far as they have a common theme, the six essays served up here under the opaque title *Science à la Mode* may be regarded as answers to this enduring question.

The essays, four of which have been published previously, consider modern cosmology (twice), geodesics, entropy, nuclear winter and the career of Evariste Galois. The first four are mostly high-level vulgarization. The last two criticize scientists who play to the public, bend to politics or write poor history. Even when popularizing, however, Rothman stops to admonish his colleagues (he is a cosmologist) for coveting their own hypotheses and evolutionary biologists for misusing ideas (the doctrine of entropy) they do not understand. Many examples of both sorts of self-deception come to mind. The tychonic system, the theory of phlogiston, the electromagnetic ether and light conceived as waves or particles, or both, or neither, have had their over-fond exponents; and physicists themselves have helped to cheapen their ideas by advertising parallels between quantum theory, depth psychology and Eastern mysticism.

It is not self-deception, however, but wilful misrepresentation of technical concepts or conscious relaxation of professional standards against which Rothman aims his two most useful essays. One records attempts to evaluate the claims made by Carl Sagan's group about the likelihood that man will exterminate himself and herself by throwing up enough smoke in a nuclear war to shut out the light of the Sun. Rothman faults the trumpeters of this dusty eschatology on two counts: their shaky numbers and hypotheses do not authorize the conclusion that we can do for ourselves what Alvarez's meteor may have done for the dinosaurs; and, by shifting attention from the colossal number of direct and immediate casualties from any serious nuclear exchange, they encourage pursuit of technical palliatives to the twilight rather than urgent efforts to remove the arsenals that might cause it. Rothman has no patience with colleagues who doubted the reliability of the wintry calculations but refused to say so, thinking that the prospect of the end of the human race might promote the cause of dis-

armament. Here the problem of the utility of deception re-presents itself. Rothman's solution is that honesty is the best policy.

The essay on Galois corrects the story commonly told by scientists of a young unappreciated genius, persecuted for his political opinions and killed in a duel arranged by police agents. Galois was a trouble maker, his genius was recognized, and he died for love not revolution. (The historian could supply hundreds of similar cases in which scientists have neither tried nor wanted to get the story straight; a short list appears in Franz Stulhofer's recent book, *Lohn und Strafe in der Wissenschaft: Naturforscher im Urteil der Geschichte*, published by Böhlau, Vienna,

in 1987.) Rothman condemns the mis-representers of Galois for suspending their usual standards of research and for deceiving their students in order to parade a picture of brilliance destroyed by functionaries dumb to the calls of genius. Thence it is an easy step to the caricature that everyone is wrong and stupid but the creative scientist. The caricature may have its tactical uses, but it is bad strategy. Rothman rightly condemns it. "This is a presumption of the highest arrogance [he writes]. Scientists should not be so enamored of themselves." □

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Cellular update

Ron Laskey

Molecular Biology of the Cell, 2nd Edn.

By Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts and James D. Watson. Garland:1989. Pp. 1218 + index. Hbk \$51.95; pbk £24.95.

The Problems Book. By John Wilson and Tim Hunt. Garland:1989. Pp.354 + index. Pbk \$14.95, £8.

THE first edition of *Molecular Biology of the Cell* appeared in 1983 and marked a milestone in the publication of textbooks in the life sciences. In order to cover the full range of cell biology, a team of authors was involved, but the material was integrated in such a way that it read as the work of a single hand. The book's success was immediate, and its appeal—to novice students, university teachers and specialized researchers—remarkably broad.

But six years is a long time, especially in such a fast-moving field as cell biology. So for some time there has been an impatient clamour for a second edition, and there is no doubt that an up-dated version was worth writing. The only question left for a reviewer is "Does it match the standard of its predecessor?". In my opinion the answer is "No". It doesn't match the first edition. It surpasses it.

A first glance at the "Contents in Brief" could mislead the reader into thinking that the second edition is almost the same as the first. There is only one entirely new chapter (an excellent one on cancer) and the old chapter on the cell nucleus has been split in two to generate a separate discussion of control of gene expression. Apart from these changes and some re-ordering of the contents, the book at first sight looks much the same as before.

It is only on starting to read that one realizes how radical the revision has been. Overhaul has not simply involved the addition of new examples, but thorough restructuring of the conceptual framework.

In all those cases I can judge, the improvement is substantial. The illustrations are another area of improvement, the explanatory diagrams and dramatic micrographs working in harmony with the text. Here is a commendably up-to-date and accurate account of the best of cell biology.

The advent of the accompanying problems book by John Wilson and Tim Hunt is a further bonus, especially for students. The book follows the main text and allows readers to test their comprehension of facts and concepts through several levels of question. The questions range from filling in the blank in a sentence or identifying a statement as true or false, to solving complex problems, which are often based on real experiments from the literature.

One matter continues to puzzle me. How have the authors managed to pack in so much new information (the developments in cell-cycle control and many other topics besides)? The length of the book is much the same as before, and there has been no sacrifice of the lucid, accessible style that marked the first edition.

So is the book really perfect? No, of course not. It is possible to identify a few niggles, such as a wrong page number in the index and oversimplified 'true or false' questions in *The Problems Book*. There are also minor points on which I disagree with the authors' emphasis or interpretation. But the overall standard of the new edition is so overwhelmingly excellent that I have no reservations in pronouncing it the best book of its kind—another milestone in biological education. □

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● An English translation of Anatole Abragam's autobiography, *Time Reversal*, is being published by Oxford University Press, priced at £25, with a release date at the end of August. The original French version of the book was reviewed in *Nature* last year. (See *Nature* **333**, 126; 1988).

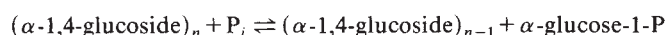
The allosteric transition of glycogen phosphorylase

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The crystal structure of R-state glycogen phosphorylase *b* has been determined at 2.9 Å resolution. A comparison of T-state and R-state structures of the enzyme explains its cooperative behaviour on ligand binding and the allosteric regulation of its activity. Communication between catalytic sites of the dimer is provided by a change in packing geometry of two helices linking each site with the subunit interface. Activation by AMP or by phosphorylation results in a quaternary conformational change that switches these two helices into the R-state conformation.

ALLOSTERIC proteins are characterized by their possession of distinct binding sites whose affinities for certain ligands depend on indirect interactions between these sites. These proteins have vital roles in metabolic regulation and receptor response. Glycogen phosphorylase *b* was the first allosteric enzyme to be isolated and analysed in detail¹. The enzyme catalyses the first step in the intracellular degradation of glycogen,



and is regulated both by allosteric interactions and reversible phosphorylation². This regulation can be understood in terms of an equilibrium between several conformational states³, ranging from a T-state with low affinity to an R-state with high affinity for substrate and activators, in which quaternary structure subunit interactions and tertiary structures are modulated by ligand binding. Phosphorylase *b*, the unphosphorylated form of the enzyme, is dependent on AMP for activity, and this activity is inhibited by glucose-6-phosphate (G6P) and ATP (Fig. 1). The enzyme shows homotropic cooperative effects (that is, interactions between identical ligands) for binding of substrates and AMP (Hill coefficients 2 and 1.4, respectively⁴) as well as heterotropic effects (that is, interactions between different ligands). The enzyme, for example, shows a 15-fold increase in affinity for phosphate as the concentration of AMP is increased from 0.015 to 0.5 mM (ref. 5). Physiological activation by hormonal or neuronal signals stimulates the conversion of phosphorylase *b* (GPb) to activated phosphorylase *a* (GPa) by kinase-catalysed phosphorylation of a single serine residue, Ser 14.

In the absence of effectors, GPb is a dimer composed of two identical subunits each having a relative molecular mass of 97,434. Activation by AMP or phosphorylation produces a change in the oligomeric state of the enzyme: it becomes largely tetrameric. Tetramers have only 12–33% of the full phosphorylase activity, but may be dissociated by glycogen or oligosaccharides to give fully active dimers^{6–8}. A substantial amount of phosphorylase *in vivo* is bound to glycogen particles^{9–11}, and the dimer is considered to be the physiologically active form of the enzyme².

High-resolution X-ray structures have been solved for T-state GPb (in the presence of a weak activator, IMP^{4,12}), and T-state

GPa (in the presence of an inhibitor, glucose^{13,14}) (Fig. 1). Comparison of both structures¹⁵ revealed the conformational changes resulting from Ser-14 phosphorylation. Communication of these changes to the catalytic site was restricted by crystal lattice forces that restrain both structures in the T-state. Here we report the structure of R-state GPb determined by molecular replacement methods, using as the search object the refined (1.9 Å resolution) T-state model of GPb (ref. 16, and K. R. Acharya *et al.*, unpublished results). As reported previously^{17–19}, monoclinic crystals of GPa, GPb or GPb bound with AMP can be obtained in the presence of ammonium sulphate (1 M). Under these conditions, GPb displays R-state characteristics such as catalytic activity, enhanced affinity for AMP, a reduced Hill coefficient, reversal of inhibition by G6P and glucose^{20,21}, and the association of dimers to form tetramers. Crystallographic studies show that sulphate mimics phosphate by binding to the catalytic site, the AMP effector site and the Ser-phosphate site, resulting in localized changes in tertiary structure. In the transition from the T- to the R-state, the two subunits of the functionally active dimer rotate about an axis perpendicular to the 2-fold symmetry axis to accommodate these changes in tertiary structure. The correlation between the tertiary and quaternary structure provides an explanation for the cooperativity of substrate and effector binding, and their mutual interdependence.

Structure determination

Crystals of rabbit muscle R-state GPb were obtained in ammonium sulphate (1 M) and β -glycerophosphate (10 mM, pH 7.5) as described^{17,18}. Native data, to a resolution of 2.9 Å, were collected at the Synchrotron Radiation Source, Daresbury on an Arndt-Wonacott oscillation camera. Strong low-resolution reflections were recorded in a 3.5 Å data set, which was subsequently incorporated into the 2.9 Å data set (Table 1).

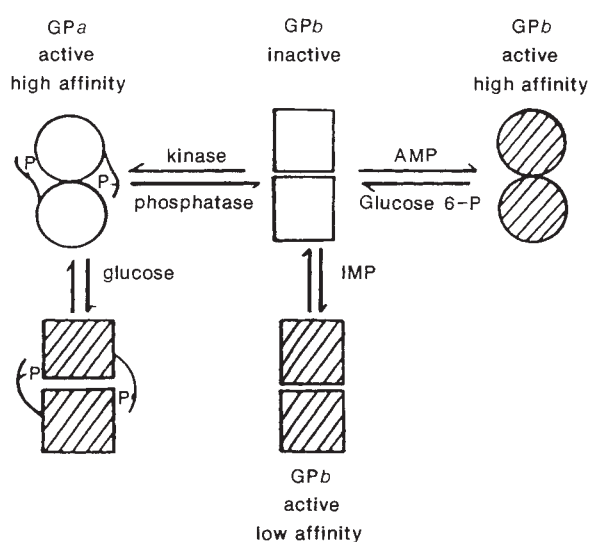


FIG. 1 A simplified representation of the allosteric and covalent activation of glycogen phosphorylase. T and R subunits are shown as squares and circles, respectively. The states referred to in the text are shaded.

The structure was determined by the molecular replacement method²², using the T-state monomer as a search object. Rotational parameters for each subunit were determined, and the translation vectors relating crystallographic and non-crystallographic subunits obtained using the Crowther and Blow translation function²³. Rigid body positional refinement, first with individual subunits and then with individual domains, was performed using the program CORELS²⁴. The resultant structure was subjected to a round of atomic positional refinement by the method of simulated annealing with the XPLOR program²⁵ using a CONVEX C210 supercomputer. Following convergence, a $2F_o - F_c$ electron density map was averaged according to the geometric relationships of the four subunits. The map obtained indicated new positions for parts of the structure which underwent large conformational changes. One subunit was manually rebuilt into the averaged density and a tetramer constructed from it. Least-squares refinement with energy restraints was then used. Non-crystallographic symmetry restraints were initially applied and gradually relaxed as the *R*-factor dropped. The final crystallographic *R*-value was 0.177.

Description of overall structure

Both R- and T-state structures possess a similar topology. The enzyme is an α - β protein with the polypeptide chain folded into two domains; the N-terminal domain of amino-acid residues 1-484 and the C-terminal domain of residues 485-842. In addition to the recognition site for the 5'-phosphate of pyridoxal phosphate (PLP)²⁶, the enzyme has three other recognition sites for the phosphate: (1) substrate (site ~ 5 Å from the cofactor phosphate); (2) AMP (site ~ 30 Å from the catalytic site); and (3) Ser-phosphate (site ~ 12 Å from the AMP site). Examination of the R-state structure shows that each of these three phosphate-recognition sites are occupied by sulphate (Fig. 2).

When two GPb dimers associate, the four subunits arrange in a planar array obeying molecular 222 symmetry (Fig. 2). The α -carbon (C_α) positions of all subunits deviate from the mean

by a root-mean-square (r.m.s.) of 0.3 Å, indicating that the four subunits are similar within the limits of data precision. The interface that is formed because of the association of the dimers to tetramers comprises residues from the 'catalytic' face of the subunit so that access to the catalytic site is slightly impeded. The involvement of a key residue from the glycogen storage site (Glu 433)²⁷ in a hydrogen bond at this interface explains why glycogen promotes dissociation of tetramers to dimers. The details of the contacts at the tetramer interface will be described later. The subunit interface of the dimer is composed of two main contact regions occurring on opposite sides of the enzyme molecule (Fig. 3). One is formed between the cap' region (residues 35'-46'), a loop connecting the $\alpha 1'$ and $\alpha 2'$ helices with the $\beta 7$ strand, and the $\alpha 2$ helix (residues 47-78) of the opposite subunit (residues from the symmetry-related subunit are designated by primes). This interface includes the AMP and Ser-phosphate binding sites. The other contact is formed by the antiparallel association of the two symmetry-related tower helices, $\alpha 7$.

A least-squares superimposition²⁸ of the R-state subunit onto the T-state subunit reveals the extent of the tertiary conformational differences between the two states. The overall r.m.s. deviation in C_α positions is 1.3 Å, although that for a core of 80% of the C_α atoms is within 0.7 Å. Those regions that deviate significantly between the two structures are either important for the allosteric response, (the tower helix), or form part of the new tetramer interface (helices $\alpha 12$, $\alpha 13$ and $\alpha 23$ - $\alpha 27$). Relative movements between the two domains do not occur.

An extensive quaternary structural change is observed such that after superimposition of one subunit of the T-state dimer onto a subunit of the R-state dimer, the r.m.s. deviation in C_α positions for the two dimers is 6 Å. This rearrangement consists of a 10° rotation of one subunit relative to the other about an axis that is approximately perpendicular to the 2-fold axis of the dimer and that intersects the dimer axis close to the region where the cap' links to the $\alpha 2$ helix (Fig. 3). The rotation draws

TABLE 1 R-state GPb data collection, processing and refinement statistics

Crystal parameters

Space group $P2_1$ $a=119.0$ Å, $b=190.0$ Å, $c=88.2$ Å, $\beta=109.35^\circ$
One tetramer, relative molecular mass of 390,000 per asymmetric unit

Data collection and processing parameters

Data set	No. crystals used	No. measurements	No. independent reflections	* R_{sym}	Percentage with $I > 3\sigma_I$	Percentage complete
2.8 Å Native	10	86,341	59,294	0.12	64	81
3.5 Å Native	1	51,459	30,319	0.075	66	75
2.8 Å and 3.5 Å native	11	131,558	64,860	0.13	65	88
4.2 Å TAMM†	3	40,133	22,983	0.045	84	88

Refinement parameters

No. protein atoms=26,816 Number of sulphate atoms=60
No. observations, $F > \sigma_F$, 8.0-2.9 Å=57,293
 $\dagger R_{\text{cryst}}=0.177$
r.m.s. deviation of bond length from ideality=0.018 Å
r.m.s. deviation of bond angles from ideality=4.0°
No water molecules were included in the refinement

Data were collected on the Wiggler station PX9.6 of the SERC Synchrotron Radiation Source, Daresbury, UK. The source was operated at an energy of 2 GeV and current ranging from 250 to 150 mA, wavelength=0.88 Å. Typical crystal dimensions were $1.0 \times 0.5 \times 0.3$ mm³. Spindle oscillation range was 1.0° for the 2.8 Å data set and 2.0° for the 3.5 Å and 4.2 Å data sets. Films were scanned on a Joyce-Loebl Scandig 3 microdensitometer and the intensities integrated by profile fitting with a modified version (D. I. Stuart, unpublished data) of the MOSCO data processing program⁴⁵ using a PDP/11 computer. Interpack scaling, partial reflection summation and data reduction were performed by the AGROVATA and ROTAVATA programs, Daresbury CCP4 program suite. TAMM derivative crystals were prepared by soaking in a solution of TAMM (0.5 mM) for 3 h. The TAMM data set was scaled to the native data using an anisotropic temperature scaling function (program ANISOSC, CCP4 suite) giving a mean fractional isomorphous difference of 12.0%. The five major heavy atom sites, derived from a difference Fourier map calculated using the TAMM and native amplitudes with calculated phases, were consistent with those assigned earlier from a 6 Å difference Patterson map⁴⁶ and suggested binding of a tetrameric compound. Four of these sites are associated with Cys 783, a cysteine that was reactive in the T-state structure⁴⁷; a fifth site was to Cys 495 of the second subunit.

* $R_{\text{sym}} = \sum_i \sum_h |I(h) - \bar{I}(h)| / \sum_i \sum_h I(h)$, where $I(h)$ is the i th measurement of reflection h and $\bar{I}(h)$ is the mean of the intensity.

† TAMM, tetrakisacetylmercurialmethane

$\dagger R_{\text{cryst}} = \sum_h |F_o - F_c| / \sum_h F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection h

the two subunits together at the cap'/ α 2-helix contact region by 1 Å and moves them apart at the tower-tower interface region by 3 Å. Details of the changes in subunit contacts are given in Table 2.

Structural changes at the tower-tower interface

The tower helix (residues 262–276) consists of a protuberance from the main body of the enzyme. It is linked to the preceding β -sheet, (residues 237–247) by a region of flexible polypeptide chain (residues 252–258). Residues 250–275 exist autonomously from their own subunit, making sparse interactions with it. The helix is anchored at its base by a short parallel β -sheet formed between residues 276–279 and 162–164. From here the polypeptide chain leads to the reverse loop of residues 281–287 (280s loop), which form a gate to the catalytic site and lead into the α 8 helix (residues 289–314).

In the T-state, an interface is formed by the antiparallel association of the two tower helices, creating a cluster of hydrogen bonds between Asn 270 and Asn 274 and their symmetry-related counterparts (Fig. 4). The unfavourable packing angle of -20° (ref. 29) is achieved by unwinding and distortion of the helices between residues 270–273. Tyrosine 262 and Ile 263 pack against Pro 281' and Tyr 280' of the symmetry-related subunit, respectively. On transition to the R-state, the geometric disposition of the helices is dramatically altered following an increase in the inclination of the helix axes to an orthodox -80° . The tilting of the two helices brings Val 266, Ile 267 and Asn 270 into a nonpolar interaction with their symmetry equivalents on the opposite subunit. The helices slide relative to each other by two turns of helix. Tyrosine 262 and Ile 263 no longer pack against Pro 281' and Tyr 280'.



FIG. 2 Ribbon representation of C_α atom trace of the R-state GPb tetramer indicating the molecular 222 symmetry. Sulphate ions are shown in yellow and the phosphate of pyridoxal 5'-phosphate in purple. The dyad axis to the T-state GPb dimers is horizontal. The dimer interface formed by the association of the two dimers is vertical. This interface involves contacts between residues from the glycogen storage site (prominent loop representing the β 15– β 16 turn) and from the bundle of helices α 23, α 24, α 26, residues 724–767.

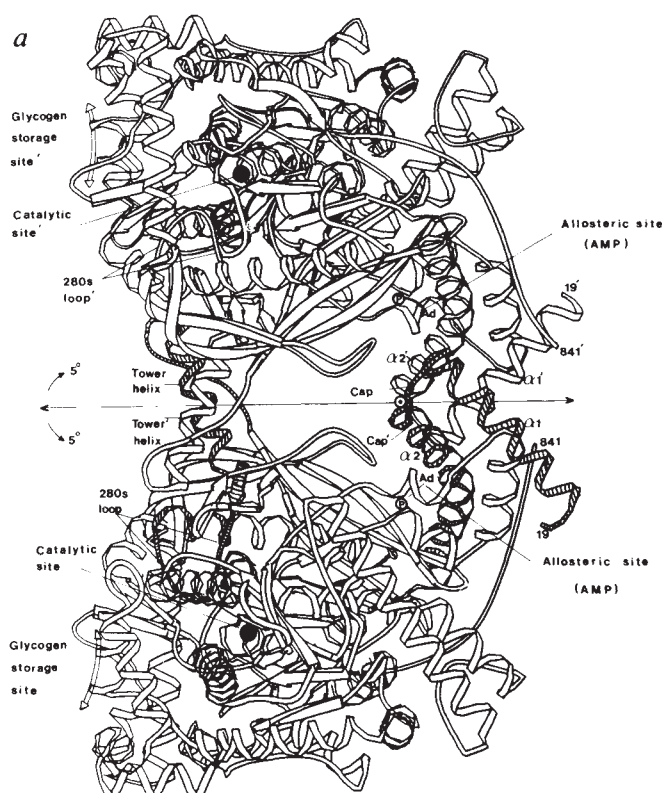
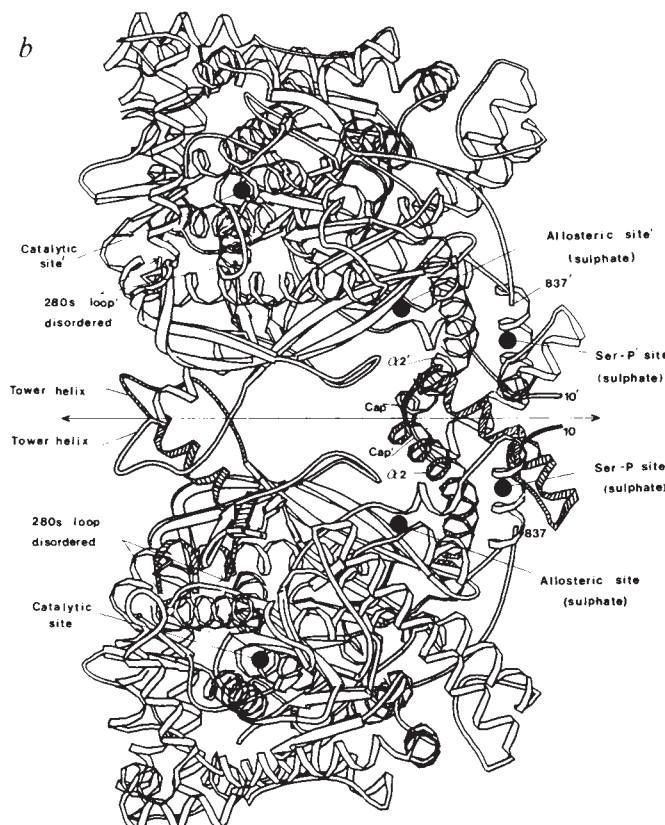


FIG. 3 Ribbon representation indicating the structural elements of: a, T-state, and b, R-state phosphorylase dimer, viewed perpendicular to the molecular dyad axes. The view is rotated 20° about the horizontal 2-fold axis of Fig. 2, and represents the yellow and purple subunits of Fig. 2. Structural elements α 1-cap- α 2, the tower helix-280s loop and the start of α 8 are indicated by shading in the lower subunit. The allosteric effector site and Ser-phosphate site are situated at the cap'/ α 2 interface. The catalytic site



is close to the 280s loop at the termination of the tower helix. The glycogen storage site, to which phosphorylase dimers are attached to glycogen particles *in vivo*, is situated on the 'catalytic face' of the molecule and forms a dimer-dimer contact in the tetramer. On transition to the R-state, each subunit rotates by 5° about an axis positioned close to the cap'/ α 2 interface (marked by $\odot$ in Fig. 3a). Ribbon diagram by a program by J. B. Priestle.

The change in helical cross-over angle is a consequence of both a quaternary and a tertiary structural change, involving a 20° tilt of the helix as an approximate rigid body relative to the remainder of the enzyme. A superimposition of the R- and T-state tower helices gives an r.m.s. deviation of 2 Å in C_α positions. This superimposition shows that the largest difference occurs between residues 272–277, and because Val 278 of R- and T-state subunits superimpose, indicates that the change in tilt of the tower helices involves adjustments in main-chain torsion angles and hydrogen bonds within the last turn of the helix. This causes related changes in the hydrogen bond pattern of the β-sheet formed between residues 276–279 and 162–164. In response, residues 162–165 twist, displacing Ile 165 by 1.5 Å. This displacement directly perturbs Asn 133 and Pro 281, transmitting a structural change to the catalytic site (Fig. 5). Thus a conformational change of the tower helix mediates transmission of a conformational response from the subunit interface to the catalytic site.

Structural changes at the catalytic site

Numerous binding studies with substrates and substrate analogues in the T-state enzyme^{30,31} have located the catalytic site ~15 Å below the molecular surface at the base of a narrow tunnel formed between the interface of the two domains, and close to the essential cofactor PLP. An inhibitory site situated

at the entrance to the catalytic-site tunnel is composed of two hydrophobic residues, Phe 285 and Tyr 613. At high concentrations AMP, IMP and other related planar non-polar molecules intercalate between these two residues, stabilizing the 280s loop and maintaining the enzyme in the T-state conformation. In the T-state, Arg 569 resides within a very buried environment, forming hydrogen bonds to the main-chain carbonyls of Asn 133, Pro 281 and Lys 608 and the side chain of Asn 133, with the guanadinium group ~7 Å from the catalytic site (Fig. 5b). In the absence of other ligands, phosphate does not bind to the catalytic site in the T-state^{31,32}.

On transition to the R-state, conformational changes at the catalytic site occur to convert it into a form capable of binding phosphate. This represents the essential feature of the allosteric transition; affinity of the catalytic site for phosphate is 15 times higher in the R-state than in the T-state^{5,33}. The presence of a phosphate-binding site in the R-state is indicated by a strong isolated feature in the electron density map to which a sulphate of crystallization can be fitted (Fig. 5). The recognition site is created by adjustments in the torsion angles in the Arg 569 side chain, shifting the guanadinium group by 7 Å so that it swings into the catalytic site and displaces Asp 283. All other residues participating in the binding site are available in the T-state. Motion of Arg 569 is triggered by the displacement of Asn 133 and Pro 281, induced by motion of Ile 165, weakening interac-

TABLE 2 Residues involved in intersubunit van der Waals contacts, salt bridges and hydrogen bonds in the R-state and T-state structure

van der Waals interactions of interface residues <4.0 Å			Salt bridges and hydrogen bond of interface residues <3.5 Å	
Residue	T-state	R-state	T-state	R-state
N-terminal tail/α5–α6 loop' interface			N-terminal tail/β5–β6 loop' interface	
R10		L115', G116'		R10:NH2–G116':O
I13		L115'		
Cap/α2' and N-terminal tail' interface			Cap/α2' and N-terminal tail' interface	
N32		Q12', I13'	H36:NE2–D838':OD2	N30:OD1–R33':NH1
R33	R33'	L18', R33'	N44:OD1–WAT–N72':OE1	R33:NH1–D61':OD1
L35		I13'		D42:OD2–Q72':NE2
H36	G65' I68', E839', F37'	I13', S14', V15'		R43:NH1–SUL':O2
F37	E839', H36', D61', V64'	L18', D61', V64'		R43:NH1–SUL':O4
V40	V64'	W67'		R43:NH2–I13':O
K41	I68'	I68'		R43:NH2–SUL':O3
N44		Q72'		
Y51		R10', I13'		
Cap/β7' interface			Cap/β7' interface	
T38	K191'	K191'	L39:O–K191':NZ	L39:O–K191':NZ
K41	R193'	R193', F196'	L39:O–R193':NH2	L39:O–R193':NH2
			L40:O–R193':NH1	L40:O–R193':NH2
			K41:NZ–E195':OE1	K41:NZ–E195':OE1
β6/β7' interface				
Y185	P194'			
Tower/β6' interface			Tower/β6' interface	
E177	L254'		E178:OE2–N250':ND2	
E178	N250'		A179:N–N250':O	
			D180:N–WAT–Y262':OH	
			D181:OD1–WAT–R269':NH1	
Tower/Tower' interface and tower/β5' interface			Tower/tower' interface	
Y262	F166', P281'	N264'		
I263	Y280'	V259'		
V266	F163', V278', Y262	L267', N270'	N270:ND2–N274':OD1	
L267	L291'	V266'	R277:NH1–N274':OD1	
R269	F163'	N270', R277'		
N270	N270', R277'	V266', R269'		

The surface area made inaccessible to solvent on formation of the subunit–subunit interface is 105 Å² larger for the T-state dimer than for the R-state dimer (2,322 Å² and 2,217 Å², respectively). Residues are identified by the one-letter code. Residues of the opposite subunit are designated by a prime. WAT, ordered water molecules in the T-state structure; SUL, sulphate. Assignment of secondary structural elements are for the T-state structure³⁰.

tions to Arg 569, and by the concerted motion of Asp 283. There is no visible electron density to account for residues 282–286 and it is assumed that these residues become mobile. Disorder of the 280s loop suggests that the gate to the catalytic site is removed, allowing oligosaccharides to bind and perturbing the inhibitor site, thus abolishing inhibition by nucleosides. Histidine 571 rotates about its C_{α} – C_{β} bond, following rupture of a hydrogen bond to Asp 283 made in the T-state, and forms a new hydrogen bond to Tyr 613.

The binding site for the phosphate of the substrate, formed from the side chains of basic residues Arg 569 and Lys 574, and the main-chain nitrogen of Gly 135, is situated adjacent to the phosphate of PLP. The phosphate of PLP and the sulphate are separated by 5 Å and a hydrogen bond is formed between the two anions. The contacts to the phosphate of the PLP are essentially the same in the T-state²⁶, namely hydrogen bonds to Lys 568, and the main-chain nitrogens of Thr 676 and Gly 677. The sulphate-binding site observed in the R-state is similar to

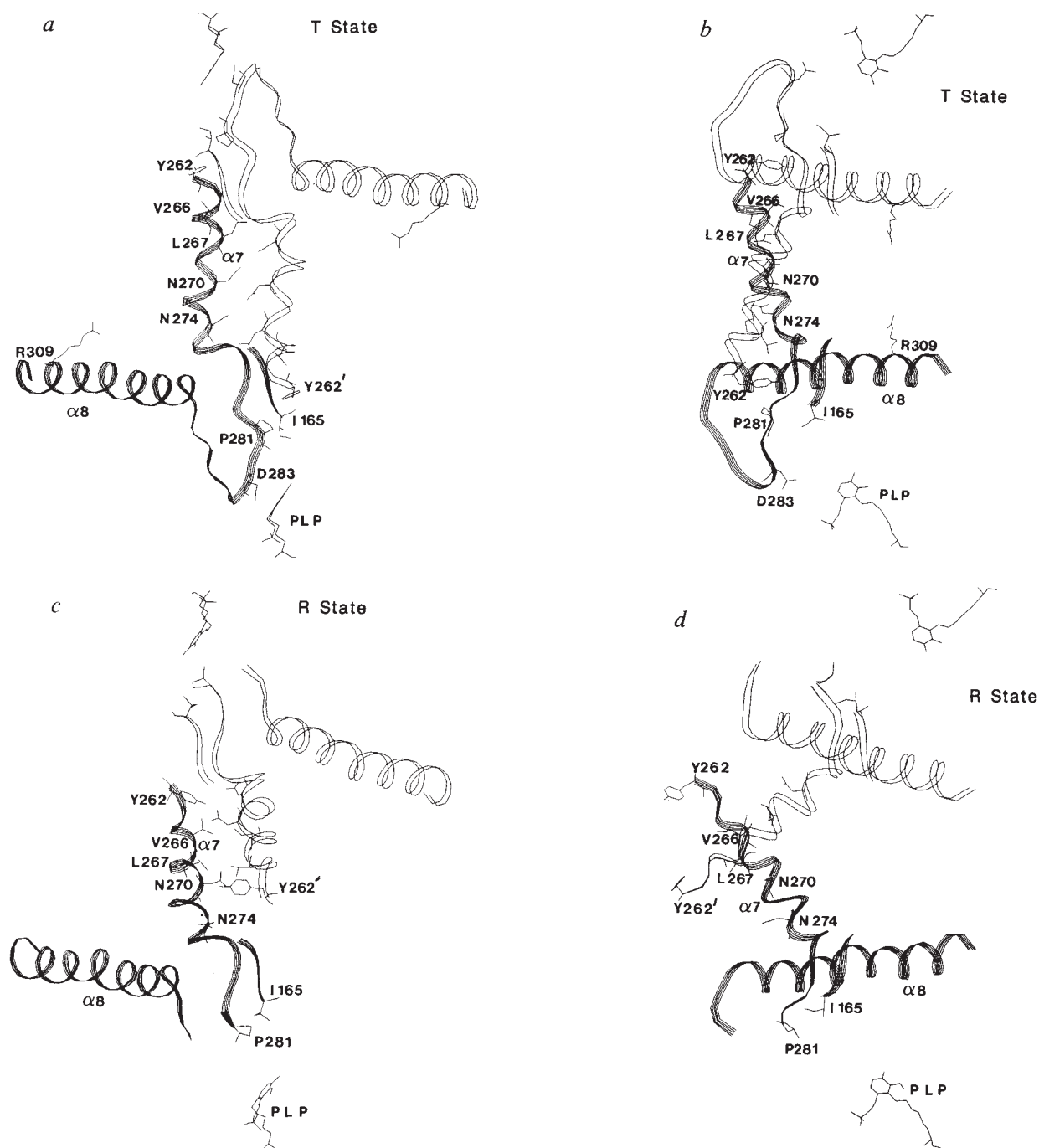


FIG. 4 Two orthogonal views showing the change in association of the symmetry-related tower helices ($\alpha 7$) on transition from the T-state (Fig. 4a,b) to R-state (Fig. 4c,d). The view in a and c is along the 2-fold axis of the T-state dimer. The view in b and d is perpendicular to the 2-fold axis and similar to that in Fig. 3. The ribbon traces residues 262 to 314 (T-state), residues 262 to 281 and 287 to 314 (R state), and residues 162 to 165 in both structures. These residues include the tower helix, the short parallel sheet between 276 to 279 and 162 to 164 and the $\alpha 8$ helix forming part

of the rigid core, which superimpose in the lower subunits of both structures. Arginine 309, which contributes to the AMP phosphate recognition site, is included for reference in $\alpha 8$ of the T-state. The catalytic site is represented by PLP. Residues displayed are Ile 165, Tyr 262, Val 266, Leu 267, Asn 270, Asn 274, Pro 281, Asp 283 (T-state only), Arg 309 and Lys-PLP 680. The change in the cluster of Ile 165, Pro 281 and Tyr 262' on T- to R-transition is apparent. For further details see text.

the phosphate-recognition site created in T-state GPb crystals when they bind the transition-state analogue, heptulose-2-phosphate^{31,34,35}. Similar movements of Arg 569 are also observed. The glucosyl-binding site for the non-reducing end of a glycogen substrate is completely formed in the T-state structure and no change occurs on transition to the R-state.

The structural data presented here were anticipated by biochemical and spectroscopic studies. Arg 569 has been shown to be unreactive to phenyl-glyoxal reagents in the T-state, but reactive in the R-state and protected by glucose-1-P^{36,37}. These observations are consistent with the burial of Arg 569 in the T-state, exposure in the R-state, and burial on the ligation of phosphate when binding glucose-1-P. The closeness of the 5'-phosphate of PLP to the substrate phosphate was indicated from the results of pyridoxal reconstitution studies of phosphorylase³⁸. It has been shown by ³¹P-NMR measurements that the ionization of PLP is sensitive to the activation of phosphorylase^{39,40}.

On the transition from the T- to R-state, the phosphate of PLP converts from a mono-anion to a di-anion, and reverts to a mono-anion on ligation of the substrate phosphate. These changes can be understood by variations in the electrostatic environment on the T- to R-state conversion. In the T-state, Asp 283 is linked through two water molecules to the 5'-phosphate of PLP, favouring the mono-anionic form of the cofactor phosphate. In the R-state, Asp 283 is displaced by Arg 569, and the additional positive charge favours the di-anionic phosphate of the cofactor. Addition of phosphate or sulphate near to the 5'-phosphate promotes protonation.

Structural changes at the allosteric sites

Analogous to the tower-tower interface, structural interdependence of quaternary and tertiary changes is observed at the cap'/ $\alpha 2$ interface, although the amplitude of both changes is smaller. Movement of the cap' towards the $\alpha 2$ helix is facilitated

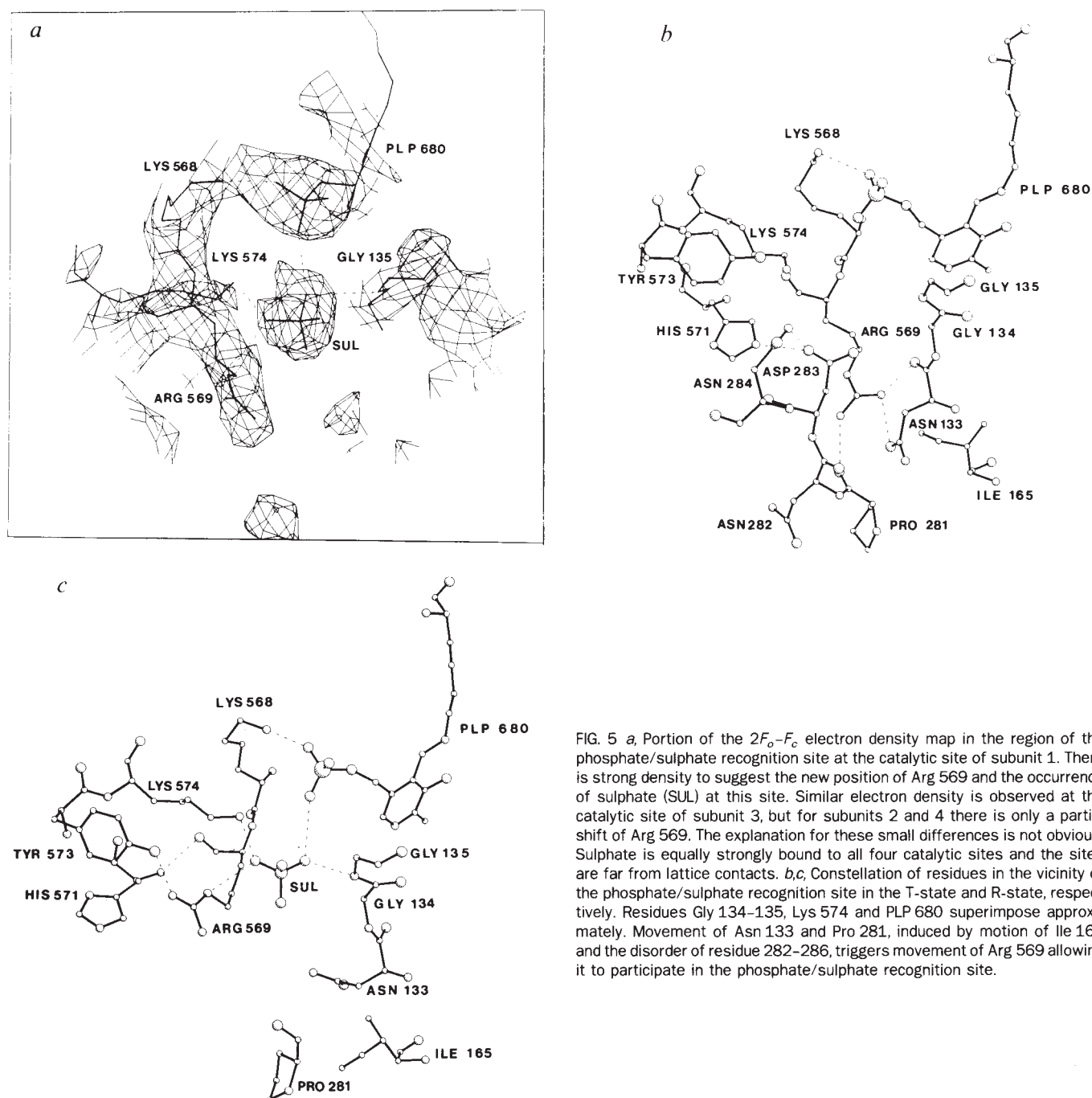


FIG. 5 *a*, Portion of the $2F_o - F_c$ electron density map in the region of the phosphate/sulphate recognition site at the catalytic site of subunit 1. There is strong density to suggest the new position of Arg 569 and the occurrence of sulphate (SUL) at this site. Similar electron density is observed at the catalytic site of subunit 3, but for subunits 2 and 4 there is only a partial shift of Arg 569. The explanation for these small differences is not obvious. Sulphate is equally strongly bound to all four catalytic sites and the sites are far from lattice contacts. *b, c*, Constellation of residues in the vicinity of the phosphate/sulphate recognition site in the T-state and R-state, respectively. Residues Gly 134–135, Lys 574 and PLP 680 superimpose approximately. Movement of Asn 133 and Pro 281, induced by motion of Ile 165 and the disorder of residue 282–286, triggers movement of Arg 569 allowing it to participate in the phosphate/sulphate recognition site.

and stabilized by concerted motions of amino-acid side-chains of these structural elements, promoted by the binding of sulphate at the Ser-phosphate site.

In the R-state crystals, sulphate is bound close (2 Å) to the Ser-phosphate 14 of GP_a, making a hydrogen bond to the side-chain of Ser 14 (Fig. 6). Residues 10–18, which are disordered in the T-state, become ordered. Rotations of the side-chains of Arg 43' and Arg 69 allow the guanadinium groups to form hydrogen bonds with the sulphate. These interactions are only favourable following the closer association of the cap' and

α 2 helix. Coupling of quaternary and tertiary changes is observed on the rotation of His 36' about the C $_{\alpha}$ –C $_{\beta}$ bond into a previously unoccupied cavity to prevent steric conflict with Ile 68 as the subunits rotate. Movement of His 36' ruptures a salt bridge to Asp 838, and residues 838–841, which are ordered in the T-state, become disordered. The position previously adopted by residues 838–841 is occupied by Arg 16. Valine 15 packs into a pocket formed between His 36' and Ile 68, and His 36' forms hydrogen bonds to the main-chain oxygen of Asn 32'.

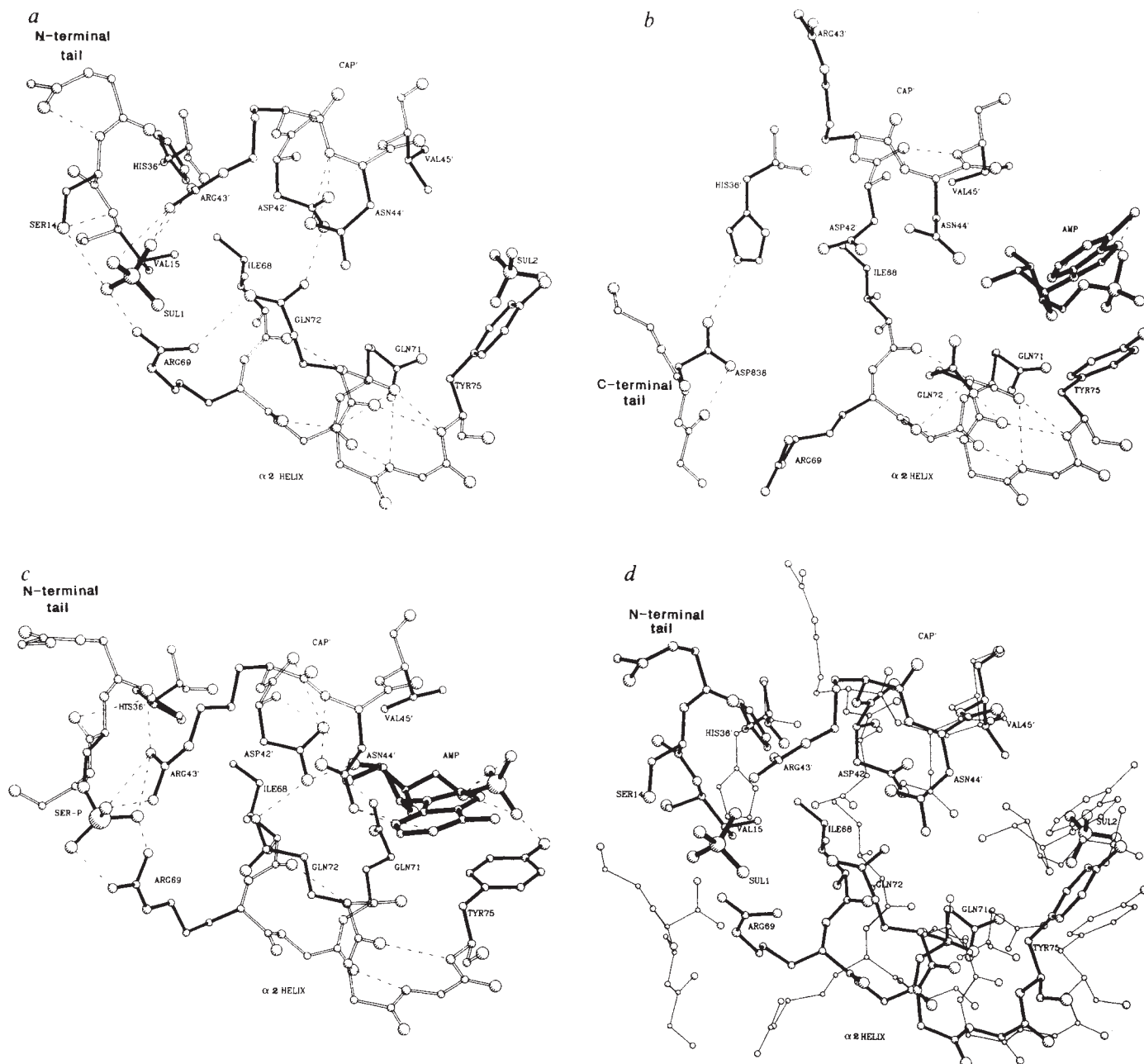


FIG. 6 Conformational changes at the cap'/ α 2 interface. *a*, GP_b R-state; *b*, GP_b T-state³⁰; *c*, GP_a-AMP complex T-state⁴⁸, and *d*, GP_b R-state (bold lines), GP_b T-state (fine lines) superimposed. The cap' and α 2 helix are marked. The N-terminal tail of GP_b R-state and GP_a T-state, and C-terminal tail of GP_b T-state are on the left. The subunit carrying the cap' of T-state GP_b and GP_a have been superimposed onto the equivalent subunit of R-state GP_b and the three structures have been drawn from the same orientation. The closer association of the cap' to the α 2 helix state is stabilized by salt bridges to Ser-phosphate in T-state GP_a, or to sulphate (SUL1) in R-state GP_b from Arg 43' and Arg 69, and interactions between Asp 42' and Gln 72.

The N-terminal tail displaces the C-terminal tail, disrupting a salt bridge between His 36' and Asp 838; subsequent rotation of His 36' prevents steric conflict with Ile 68. The change in conformation of Asp 42' Asn 44', Gln 72 and Tyr 75 creates a site with high affinity for AMP in R-state GP_b and T-state GP_a. The sulphate (SUL2) bound in GP_b R-state is close to the site for the phosphate of AMP and is shown on the right of *a* and *d*. The three arginines (Arg 242, Arg 309 and Arg 310) that stabilize this site have been omitted for clarity. (The GP_a coordinates^{15,48} were provided by S. R. Sprang, R. J. Fletterick and E. Goldsmith.)

The quaternary and tertiary structural changes that occur in the transition of GPb from the T- to R-state also result in an allosteric site with high affinity for AMP located 12 Å from the sulphate/Ser-phosphate site. In the T-state structure, His 36' intercalates between Val 64 and Ile 68 of the $\alpha 2$ helix, inducing the helix to unwind by 1 Å towards its C-terminus. Rotation of His 36' on transition to the R-state allows re-ordering and contraction of the helix, positioning Gln 72 so as to form a hydrogen bond to Asp 42' of the cap'. Comparison with T-state GPb and GPa suggests that the side-chains of Asp 42', Asn 44' and Gln 72 are correctly positioned to hydrogen bond to AMP, although this has to be confirmed by direct binding studies.

Both AMP and Ser-phosphate 14, or sulphate, stabilize the R-state by interacting with residues from both subunits that are only available in the R-state conformation. Communication between Ser-phosphate 14 and the allosteric effector site is mediated through a closer association of the cap' and $\alpha 2$ helix, and re-ordering of the $\alpha 2$ helix. Thus, although the enzyme does not possess a phosphorylated serine, in the presence of sulphate the protein conformation at this interface is almost identical to that of the glucose-inhibited T-state GPa¹⁵ (Fig. 6).

Discussion

The allosteric transition of glycogen phosphorylase can be simply described. The transition from the T-state to the R-state involves small changes in tertiary structure at the ligand-binding sites and the subunit interface regions, and little change in the remainder of the subunit. These are coupled to large changes in quaternary structure that involve rotation of the two subunits with respect to one another. The change in quaternary structure directly affects the allosteric effector site and the Ser-phosphate site, which are located at the cap'/ $\alpha 2$ interface, and transmits a signal to the catalytic site and nucleoside-inhibitor site through conformational changes of the two tower helices. Movement of the tower helix indirectly leads to a replacement of an aspartate by an arginine residue at the catalytic site, and the opening of a tunnel to allow access for substrate. The crystallographic studies show tertiary structural changes in response to ligand binding⁴¹. Each of these changes includes a movement of arginine residues to create a phosphate- (or sulphate-) recognition site. The correct position of arginine side chains is coupled

to the presence of an anion at these sites. At the catalytic site, the movement of Arg 569 contributes to the stabilization of the close proximity of the substrate anion to the cofactor 5'-phosphate, which is an essential part of the enzymic mechanism. At the Ser-phosphate site, two arginines, one from each of the subunits, move to create the phosphate site, and the presence of the di-anion leads to a re-ordering of the basic N-terminal tail. The major feature in creating the high affinity allosteric site is the movement of the cap'/ $\alpha 2$ interface, but the phosphate of AMP, which is essential for activation, also induces movement of an arginine, (Arg 309) to create with Arg 310 a site with high affinity for phosphate.

The alternate packing adopted by the tower helices provides a particularly suitable method by which to couple tertiary and quaternary conformations. A rotation of the two subunits in the allosteric transition alters the relative disposition of the two helices. To achieve more favourable helix packing-geometry a further tertiary structural change occurs that affects the relative disposition of the helices. Because the geometry of one helix is constrained by that of the symmetry related helix, molecular symmetry is preserved and a concerted conformational change of both subunits occurs. A strict coupling of quaternary to tertiary structure is required so that heterotropic allosteric effectors bound at the cap'/ $\alpha 2$ interface, 35 Å from the tower helix interface, can transmit a conformational change to the catalytic site. Thus homotropic and heterotropic mechanisms involve the same gross structural changes and are mutually interdependent. Phosphorylase was one of the first enzymes whose kinetic properties were described by a two-state interpretation of the concerted allosteric model of Monod, Wyman and Changeux^{3,42}. Our account of the structural transition agrees closely with that envisaged in this model. Two quaternary conformations have been characterized by the alternative packing of the tower helices.

The change in helix packing of the tower helices is quite different from the much smaller helix-shear interface movements recognized for the conformational change observed in citrate synthase⁴³ and different structures of insulin⁴⁴. The transmission of allosteric effects through changes in helix-helix packing as seen in phosphorylase, or through helix-shear movements, may provide a basis for understanding receptor-mediated transmembrane signalling. □

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A submillisecond pulsar and the equation of state of dense matter

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IF the submillisecond pulsar in the remnant of supernova 1987A really is rotating stably with a period P_{smp} of 0.508 ms (ref. 1), its existence can be used to rule out nearly all 'realistic' equations of state for dense nuclear matter. (An alternative hypothesis, that the pulsar is vibrating rather than rotating², yields no such constraints.) We present here a simple equation of state that yields, in the non-rotating case, maximally compact models of neutron stars, and argue that stars constructed in this way will also be stable when rotating with periods < 0.5 ms. Additional constraints found by applying the same equation of state to the 'slowly' rotating pulsar PSR1913+16, whose mass is accurately known, leaves only a small range of acceptable parameters for neutron star models based on equations of state that obey the causality requirement that their sound speeds are less than the speed of light.

Rapid rotation may induce neutron-star instability (see ref. 3 and refs therein). The discovery of the millisecond pulsar PSR 1937+214 (which had the highest known rotational frequency, $\Omega = 4.03 \times 10^3 \text{ s}^{-1}$, before the announcement in ref. 1) implies some mild constraints on the parameters of possible neutron-star models³. The existence of a submillisecond pulsar with $\Omega_{\text{smp}} = 1.24 \times 10^4 \text{ s}^{-1}$, however, is expected to imply more stringent constraints.

An object of mass $M \approx M_{\odot}$ and radius $R < 10$ km that is rotating with a frequency of $\sim \Omega_{\text{smp}}$ requires a relativistic treatment of both rotation and gravity. Such a calculation has been recently done for a uniformly rotating neutron star⁴. For a rather broad set of dense-matter equations of state the following sequence of neutron-star instabilities is induced by an increasing Ω . First, the gravitational-radiation non-axisymmetric instabilities appear for the $m = 4$ and then for the $m = 3$ perturbations. Second, for a slightly higher $\Omega = \Omega_K$, the Kepler frequency is reached at the equator and mass shedding begins. Because no uniformly rotating star can reach $\Omega > \Omega_K$, the Kepler frequency sets a very rigid upper limit on the possible uniform rotation in neutron-star models. None of the equations of state studied⁴ can give $\Omega_K > \Omega_{\text{smp}}$ for the configuration with the 'standard' baryon mass, $M_B = 1.4 M_{\odot}$. Actually, the condition for the stability in the rotating neutron-star model is even stricter—instead of Ω_K , we should use a slightly lower value Ω_{lim} , corresponding to the gravitational radiation $m = 3, 4$ instabilities. In practice, however, we can approximate $\Omega_{\text{lim}} \approx \Omega_K$ (ref. 4). This approximation is particularly good for equations of state yielding models of very compact neutron stars, which are the only promising candidates for the submillisecond pulsar model.

The value of Ω_K increases with increasing M_B and reaches its maximum $\Omega_{K,\text{max}}$ for the configuration with a maximum allowable mass. $\Omega_{K,\text{max}} > \Omega_{\text{smp}}$ can only be obtained for one equation of state⁵ considered in ref. 4 but the maximum mass of a non-rotating neutron star $M_{\text{max}}(\Omega = 0)$ obtained using this equation of state is smaller than $M_{\text{PSR1913+16}} = 1.45 M_{\odot}$ (ref. 6) (PSR1913+16 can be well approximated as a non-rotating neutron star because $P_{\text{PSR1913+16}} = 59 \text{ ms} \gg 2\pi/\Omega_K$.) For the equations of state of Arponen⁷ and Friedman and Pandharipande⁸ $\Omega_{K,\text{max}} \approx \Omega_{\text{smp}}$, within $< 1\%$, and thus we should expect that $\Omega_{\text{lim,max}} < \Omega_{\text{smp}}$. We conclude that, for uniform rotation, the constraint $\Omega_{K,\text{max}} > \Omega_{\text{smp}}$ combined with $M_{\text{max}}(\Omega = 0) > M_{\text{PSR1913+16}}$ rules out all five 'realistic' equations of state of dense matter studied in detail in ref. 4. (The assumption of

uniform rotation is based on our belief that the dissipation of differential rotation in a newly born, hot neutron star occurs on a timescale much shorter than two years.)

To obtain a graphical representation of the $\Omega_K > \Omega_{\text{smp}}$ constraint in the M - R plane for the non-rotating neutron-star models, we introduce an analytical formula for $\Omega_{K,\text{max}}$ that reproduces the numerical results⁴ very well ($< 5\%$ error)

$$\Omega_{K,\text{max}} = 7.7 \times 10^3 \left(\frac{M_{\text{max}}}{M_{\odot}} \right)^{1/2} (R_{M_{\text{max}}})^{-3/2} \text{ s}^{-1} \quad (1)$$

where M_{max} and $R_{M_{\text{max}}}$ are the maximum allowable mass and the corresponding stellar radius (in units of 10 km) for the non-rotating configurations. Generally, equation (1) works extremely well for sufficiently compact configurations—which is the case for the maximum-allowable-mass configurations. Equation (1) is not based on relativistic considerations so its successful application to neutron-star pulsars is surprising.

Using equation (1), we write a necessary condition for a given dense-matter equation of state to give uniformly rotating neutron-star models that are consistent with a given observed pulsar period P (in ms),

$$\frac{M_{\text{max}}}{M_{\odot}} > 0.67 (P)^{-2} (R_{M_{\text{max}}})^3 \quad (2)$$

where the values of M_{max} and $R_{M_{\text{max}}}$ (in units of 10 km) refer to the non-rotating neutron-star models with the maximum allowable mass. Condition (2) has the same functional form as the stability conditions for the uniform rotation discussed in ref. 3. Equation (2) has, however, the practical advantage of being fitted to the results of numerical general-relativity calculations⁴ for uniformly rotating neutron-star models from a broad range of dense-matter equations of state. There is a significant

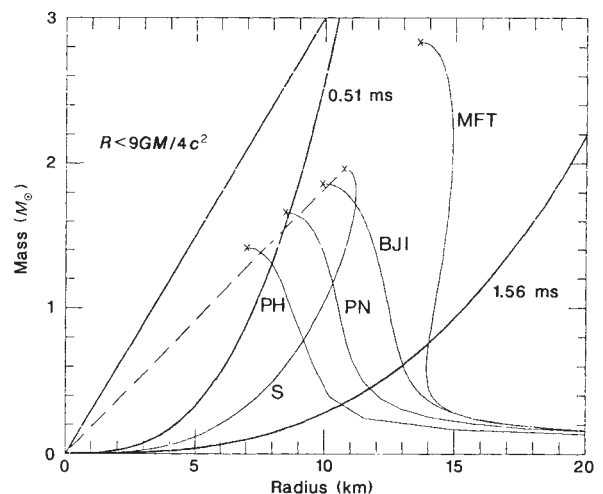


FIG. 1 Allowed regions in the M - R plane for the non-rotating neutron-star models. Configurations with the maximum allowable mass are denoted by crosses. The limiting curves for the extreme configurations have been obtained using equation (2). Equations of state for which the extreme configurations lie to the right of the solid curve marked 1.56 ms are excluded by the existence of the millisecond pulsar PSR1937+214, whereas those that yield the extreme configurations lying to the right of 0.51 ms curve are excluded by the pulsar announced in ref. 1. Configurations with $R < 9GM/4c^2$ are prohibited by general relativity (see, for example, ref. 18). The $M(R)$ curves for various equations of state are: MFT—mean-field theory, model 0.17 of Haensel *et al.*¹⁹; BJI—model I of Bethe and Johnson²⁰; PN—Pandharipande equation of state for neutron matter⁸; PH—Pandharipande equation of state for the hyperonic matter¹⁰; S—strange-quark stars for the simplest MIT bag model with $B = B_0 = 60 \text{ MeV fm}^{-3}$. The maxima of the strange-star $M(R)$ curves corresponding to $B > B_0$ lie on the dashed line connecting the maximum of the S curve with the point $M = 0, R = 0$.

difference in the numerical coefficients: our value is 0.67, compared with at least 0.88 from Table 1 of ref. 3.

In Fig. 1 we show M against R for non-rotating star models for several dense-matter equations of state. Although all 'realistic' equations of state easily satisfy condition (2) for $P = P_{\text{PSR1937+214}}$, only the 'soft' matter ones can, marginally, fulfil condition (2) for $P = 0.51$ ms. The PN model⁹ fulfils this condition only for the configurations in which the mass is very close to M_{max} , and the PH equation of state¹⁰ fails to satisfy the constraint $M_{\text{max}} > M_{\text{PSR1937+214}}$. Therefore, to satisfy the condition (2) and the constraint for M_{max} we are forced to choose the equation of state very carefully; it must be somewhat softer than the PN one, but stiffer than the PH equation of state. Even then condition (2) and the constraint $M_{\text{max}} > M_{\text{PSR1937+214}}$ allow for the existence of a very narrow range (with masses very close to the maximum allowable mass) of neutron-star models that are stable at $P = P_{\text{smp}}$.

If strange quark matter is the absolute ground state of matter at zero pressure¹¹, then we can consider the possibility of strange stars¹¹⁻¹⁴. The macroscopic parameters of strange stars, in particular their M - R relationship, are determined primarily by the MIT (Massachusetts Institute of Technology) bag-model constant B . We consider the simplest model of free massless quarks¹¹. Figure 1 shows an example of the M - R curve of the strange stars, for $B = 60 \text{ MeV fm}^{-3}$. The parameters of the maximum-allowable-mass configurations with scale with the value of B according to^{11,12}

$$\begin{aligned} M_{\text{max}} &= 1.96 \left(\frac{B_0}{B} \right)^{1/2} M_{\odot} \\ R_{M_{\text{max}}} &= 10.7 \left(\frac{B_0}{B} \right)^{1/2} \text{ km} \end{aligned} \quad (3)$$

where $B_0 = 60 \text{ MeV fm}^{-3}$, so that the extreme configurations lie on the straight line in the M - R plane

$$\frac{2GM}{Rc^2} = 0.54 \quad (4)$$

At first glance Fig. 1 seems to show that there exists a large area corresponding to models of strange-stars that can rotate at

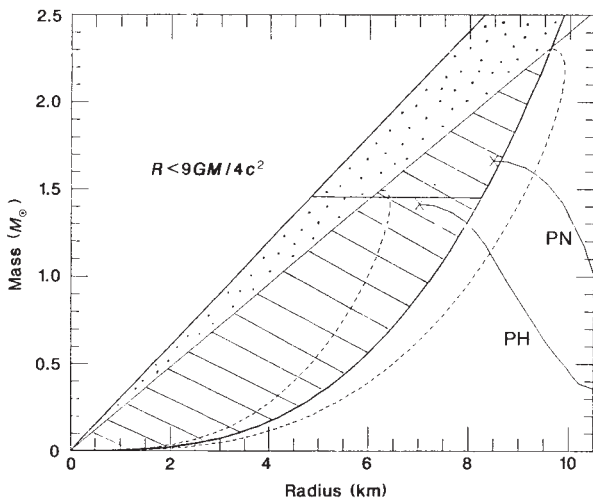


FIG. 2 Heavy lines are the boundaries of the region of extreme non-rotating neutron-star configurations allowed by general relativity ($R < 9GM/4c^2$) and by the characteristics of the pulsar announced in ref. 1 ($\Omega_{K,\text{max}} > \Omega_{\text{smp}}$). Dashed lines: the $M(R)$ curves for the self-bound, causality-limit equation of state, equation (5), for $\rho_0 = 8.4 \times 10^{14} \text{ g cm}^{-3}$ (right curve) and for $\rho_0 = 2.0 \times 10^{15} \text{ g cm}^{-3}$ (left curve). The hatched area corresponds to the causal equations of state. If the causality requirement is relaxed, the allowed region is augmented by the dotted sector. The horizontal line corresponds to $M = M_{\text{PSR1937+214}}$.

$\Omega > \Omega_{\text{smp}}$. The condition for the self-bound state of strange matter to be energetically preferred over the normal, nucleonic one, is, however, satisfied only for $B < 1.5B_0$ (ref. 14) and this excludes the possibility of $\Omega = \Omega_{\text{smp}}$ for strange stars. The inclusion, within the MIT bag model, of the finite mass of the strange quark and of the quantum chromodynamic interaction between quarks^{12,13} does not change this conclusion. We note that strange-star models are also ruled out for some of the radio pulsars, namely those exhibiting glitches¹⁵. (The mass of the solid crust of strange stars is too small ($< 10^{-5} M_{\odot}$ (ref. 13)) to account for the observed discontinuities in the pulsar timing.)

A question of practical importance arises: what is the equation of state that is optimum from the point of view of maximum stability of the uniformly rotating neutron star and that is consistent with the general physical requirements, as well as with the constraint $M_{\text{max}}(\Omega = 0) > M_{\text{PSR1937+214}}$? To obtain a massive object of maximum mean density, our equation of state should correspond to matter of maximum stiffness (velocity of sound = c) that is also very dense, even at pressure $P = 0$. This corresponds to

$$P = (\rho - \rho_0)c^2 \quad (5)$$

where ρc^2 is the energy density of matter. Our equation of state contains only one free parameter ρ_0 , which will determine the value of M_{max} . Matter described by equation (5) has a superdense self-bound state at $P = 0$, which is reminiscent of the 'abnormal' state of matter, discussed in the mid-1970s¹⁶.

The M - R curves for the self-bound, causality-limit equation of state, equation (5), are shown in Fig. 2. For $\rho_0 = 0.84 \times 10^{15} \text{ cm}^{-3}$ we get $\Omega_{K,\text{max}} \approx \Omega_{\text{smp}}$ at $M_{\text{max}}(\Omega = 0) = 2.31 M_{\odot}$. For a sufficiently high ρ_0 , all configurations are stable at $\Omega = \Omega_{\text{smp}}$. Non-rotating configurations of maximum mass depend in a very simple way on the parameter ρ_0 ,

$$\begin{aligned} M_{\text{max}}(\rho'_0) &= \left(\frac{\rho_0}{\rho'_0} \right)^{1/2} M_{\text{max}}(\rho_0) \\ R(\rho'_0) &= \left(\frac{\rho_0}{\rho'_0} \right)^{1/2} R(\rho_0) \end{aligned} \quad (6)$$

so that the maxima of the $M(R)$ curves corresponding to different ρ_0 lie on the straight line

$$\frac{2GM}{Rc^2} = 0.71 \quad (7)$$

The straight line, equation (7), is very similar to that corresponding to an absolute upper bound for the surface redshift of neutron stars¹⁷. Thus, the condition of causality (velocity of sound $\leq c$) leaves us with a rather large area of compact configurations that can rotate uniformly with $\Omega > \Omega_{\text{smp}}$ but, if we impose the additional constraint $M_{\text{max}}(\Omega = 0) > M_{\text{PSR1937+214}}$, then the permitted area for the maximum-mass configurations reduces to a rather small 'triangle'.

Having considered various possibilities for constructing very compact neutron-star models, we can conclude that the possible existence of a submillisecond pulsar with $P_{\text{smp}} = 0.5$ ms could be reconciled with a theory of neutron stars that is based on general relativity and a causal equation of state. We are then forced, however, to accept a rather narrow range of the models of dense matter that yield neutron stars having the maximum mean density, and we must reject nearly all existing 'realistic' equations of state. This statement can be significantly weakened if we consider the possibility that the 'abnormal' state of matter, which leads to very compact neutron stars, is separated from the 'normal' one, described by the standard equations of state, by a large energy barrier. Then, depending on the scenario of a neutron-star's formation, it could be in a 'normal' or an 'abnormal' state. The observation of a submillisecond pulsar may be relevant only for the 'abnormal' neutron stars, just because this pulsar is an 'abnormal' star and the condition

$M_{\max} > M_{\text{PSR1913+16}}$ could be only relevant for 'normal' neutron stars, because this binary radio pulsar happens to be a 'normal' neutron star. $\square$

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Oxygen ordering, phase separation and the 60-K and 90-K plateaus in $\text{YBa}_2\text{Cu}_3\text{O}_x$

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A PLOT of the superconducting transition temperature (T_c) against oxygen content (x) for $\text{YBa}_2\text{Cu}_3\text{O}_x$ exhibits two 'plateaus' when oxygen is removed from the material at low temperatures. T_c remains nearly constant at ~ 60 K for $x = 6.6$ – 6.7 and nearly constant at ~ 90 K for $x = 6.8$ – 7.0 . It is now common to assume that there are two distinct superconducting phases in $\text{YBa}_2\text{Cu}_3\text{O}_x$, the '60-K' and '90-K' phases, and that the two plateaus correspond to single-phase regions of the respective phases^{1,2}. $\text{YBa}_2\text{Cu}_3\text{O}_x$ samples prepared at low temperatures contain a variety of ordered oxygen superstructures^{3–5}. Several theoretical studies have tried to predict the phase equilibria between these ordered structures^{6,7} and to explain how oxygen ordering leads to the T_c plateaus^{8,9}, but no clear understanding of the interrelationships amongst these factors has yet emerged. This is partly because the techniques used previously to prepare the low-temperature samples do not control or monitor all of the key processing variables, particularly the oxygen partial pressure. Here we report on the ordered oxygen structures and superconducting properties of $\text{YBa}_2\text{Cu}_3\text{O}_x$ samples prepared in precisely controlled oxygen environments using a solid-state ionic technique. We find no evidence for phase separation between structures that have widely different oxygen content, but we do see electron diffraction evidence for phase separation between distinct phases that differ only slightly in oxygen content, and these regions of phase separation coincide with the T_c plateaus. These results show that the commonly held view that the two plateaus correspond to single-phase regions of respective 60-K and 90-K phases is incorrect: the changes in superconducting properties with oxygen content in $\text{YBa}_2\text{Cu}_3\text{O}_x$ cannot be explained on this basis.

The solid-state ionic cell used to prepare the samples is described elsewhere^{10–13}. $\text{YBa}_2\text{Cu}_3\text{O}_{7.00 \pm 0.02}$ starting material (oxygen stoichiometry measured by iodine titration) was heated to 500 °C, and then oxygen was titrated out until the desired oxygen content was reached, a process that took typically 2–3 days. The sample remained at 500 °C for a further day and was then slowly cooled to 25 °C by stepping down the furnace temperature over a 28-h period. Thus the ordered structures observed here were those that were frozen in as the temperature was slowly reduced to room temperature. These structures do not necessarily correspond to the ordered structures present at 500 °C.

Figure 1 summarizes the T_c s and ordered oxygen structures (denoted by their superlattice wavevectors) as a function of oxygen content. All of the electron diffraction patterns taken from different crystals in the same sample were essentially identical, except for the $x = 6.65$ and $x = 6.90$ samples (see below). Figure 2 is a series of line traces through electron diffraction peaks running along the a^* direction in selected samples, all taken from [001]-zone-axis patterns. The largest peaks in these traces are fundamental reflections, whereas the smaller peaks are superlattice reflections arising from oxygen ordering in the basal plane of $\text{YBa}_2\text{Cu}_3\text{O}_x$. No superlattice reflections were observed in tetragonal material. In particular, no $(\frac{1}{4} \frac{1}{4} 0)$ superlattice wavevector was found¹⁴.

The ordered structure characterized by a $(\frac{1}{2} 0 0)$ superlattice wavevector, which is often called the 'Ortho II' phase, was seen in all crystals for $x = 6.28$ – 6.61 and in some crystals for $x = 6.65$. At $x = 6.28$, this superstructure was barely discernible in 10-nm orthorhombic microdomains in a 'tweed' microstructure. At higher oxygen content, it was clearly present in a macroscopically twinned microstructure. [010]-zone-axis patterns from Ortho II crystals in the $x = 6.50$ – 6.65 samples typically showed sharp superlattice reflections (Fig. 3), indicating that there was long-range ordering of this superlattice along the c^* direction. Dark-field images using superlattice reflections showed that this ordering occurred throughout crystals with intense $(\frac{1}{2} 0 0)$ reflections (Fig. 4), and not just as the isolated microdomains reported previously¹⁵. The electrical properties of the Ortho II phase varied considerably with oxygen content, ranging from an antiferromagnetic insulator at $x = 6.28$ to a 60-K superconductor at $x = 6.61$. It is clearly a misnomer to refer to Ortho II as just the '60-K phase'.

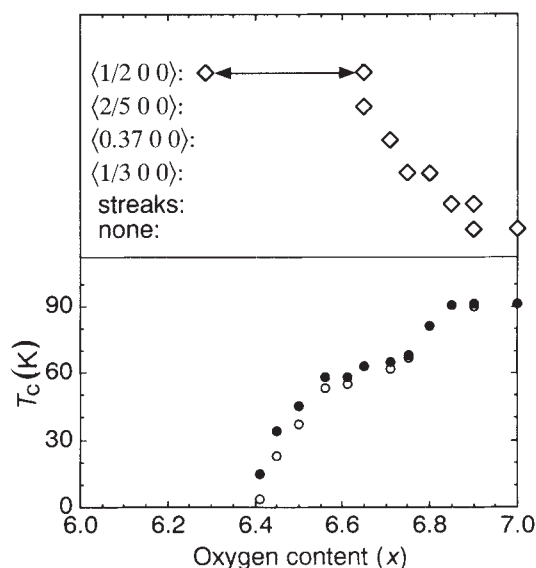


FIG. 1 Superconducting transition temperature T_c and ordered oxygen structures (denoted by their superlattice wavevectors) plotted against oxygen content. The only samples with more than one type of oxygen ordering are the $x = 6.65$ and $x = 6.90$ samples. ●, Diamagnetic onset; ○, Zero resistance.

Both the Ortho II phase and an ordered structure with $\langle \frac{2}{3} 0 0 \rangle$ wavevector were found in the $x = 6.65$ sample (Fig. 2). A progression of ordered structures occurred for $x = 6.71$ – 6.90 , and the Ortho II phase was no longer present (Figs 1 and 2). These structures, unlike the Ortho II phase, did not exhibit long-range correlation along the c^* direction. At $x = 6.90$, electron diffraction patterns for some crystals displayed very diffuse streaks along a^* (as in $x = 6.85$ sample), whereas diffraction patterns for other crystals contained no evidence for further oxygen ordering (as in the $x = 7.00$ starting material). All of the ordered structures are consistent with the removal of entire chains of oxygen along the b axis of $\text{YBa}_2\text{Cu}_3\text{O}_x$. This type of ordering minimizes the number of threefold-coordinated copper in the basal plane. Moreover, it allows for the creation of Cu^+ ions even in samples with $x > 6.5$.

These results show that the two T_c plateaus do not correspond to single-phase regions of respective 60-K and 90-K phases. If they did, only one type of ordering would be seen across each

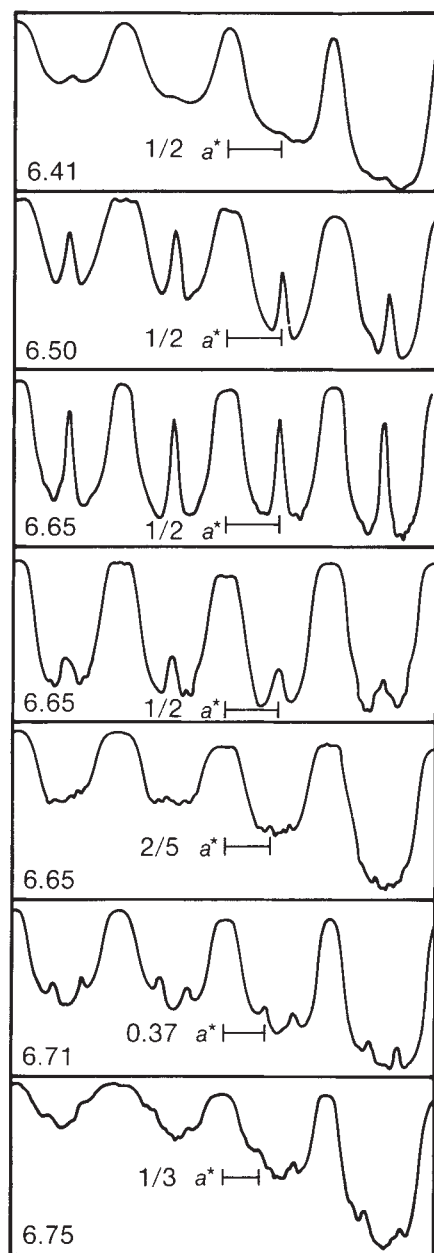


FIG. 2 Electron diffraction pattern line traces along the a^* direction showing fundamental and superlattice reflections in selected samples. The average oxygen content and superlattice wavevector for each sample are indicated.

plateau and a mixture of these two orderings would occur between the plateaus. Figure 1 shows that this is not the case. The 60-K plateau has the $\langle \frac{1}{2} 0 0 \rangle$ superlattice at $x = 6.61$, a mixture of the $\langle \frac{1}{2} 0 0 \rangle$ and $\langle \frac{2}{3} 0 0 \rangle$ superlattices at $x = 6.65$, and the $\langle 0.37 0 0 \rangle$ superlattice at $x = 6.71$. The 90-K plateau has diffuse streaks along a^* at $x = 6.85$ and no signs of further ordering at $x = 7.00$. Between the two plateaus, there is a progression of ordered structures, not a mixture of crystals with the $\langle \frac{1}{2} 0 0 \rangle$ superlattice and crystals with no signs of additional ordering. Thus, the changes in superconducting properties with oxygen content in $\text{YBa}_2\text{Cu}_3\text{O}_x$ cannot be explained simply by two separate and distinct superconducting phases corresponding to the 60-K and 90-K plateaus.

In fact, these results show that the ranges of x for which T_c is changing rapidly correspond to single-phase regions. (The sequence of ordered structures for $x = 6.7$ – 6.9 behaves like a single nonstoichiometric phase.) The T_c plateaus, however, may correspond to two-phase regions. This behaviour is consistent with that expected when the Gibbs phase rule is applied to a pseudobinary system; intrinsic properties can vary in single-phase regions, but they are fixed in the coexisting phases by virtue of the phase rule¹⁶. Finding more than one type of diffraction pattern in the $x = 6.65$ and $x = 6.90$ samples is consistent with the idea that phase separation between ordered structures with only small differences in oxygen content may be occurring at these average stoichiometries. Moreover, a recent coulometric-titration study¹⁷ supports the possibility of a low-temperature miscibility gap centred at $x = 6.65$. Several caveats need to be mentioned, however, regarding this speculation that the T_c plateaus arise from phase separation. First, at equilibrium in off-stoichiometry samples, there should be phase separation into perfectly ordered phases at low temperatures, but complete phase separation may be difficult to achieve because of limited

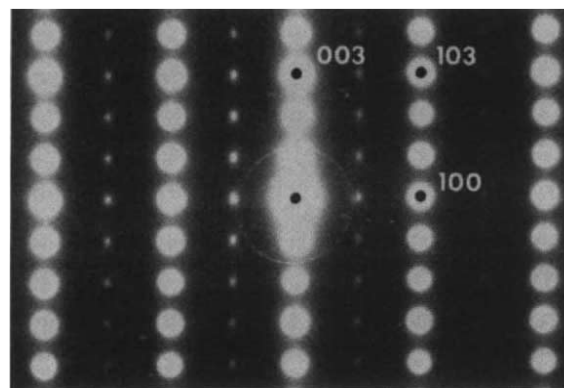


FIG. 3 Typical [010]-zone-axis pattern from the Ortho II phase, indicating that the $\langle \frac{1}{2} 0 0 \rangle$ superlattice has long-range correlation in the c^* direction.

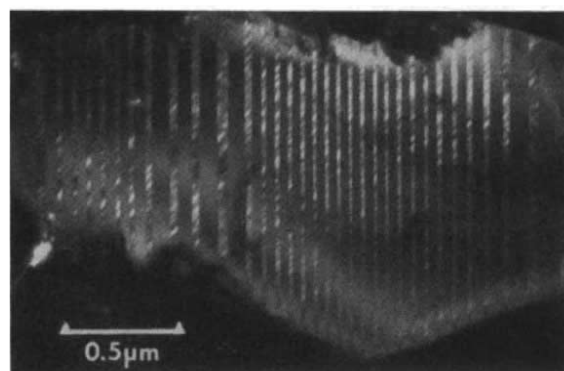


FIG. 4 Dark-field image of the Ortho II phase using $\langle \frac{2}{3} 0 0 \rangle$ superlattice reflection. Oxygen ordering occurs throughout the crystal.

oxygen mobility at low temperatures. Thus, the plateaus may be 'smeared out' in nonequilibrated samples and even absent in samples rapidly quenched from high temperatures. Second, the apparent phase separation at $x = 6.90$ could also be caused by nonuniform oxygen uptake during the slow cooling because there is an appreciable oxygen pressure in the apparatus at this stoichiometry. (This caution does not apply to the $x = 6.65$ sample.) Third, because electron diffraction examines an exceedingly small portion of each sample, it would be useful to see if complementary measurements of the structure and properties of the bulk material also provide evidence for phase separation at the same stoichiometries. We note, however, that phase separation between phases with only small differences in oxygen content may be difficult to detect using bulk techniques. Alternatively, the ordered structures and T_c plateaus could be determined in $\text{YBa}_2\text{Cu}_3\text{O}_x$ derivatives to see if similar correlations are found. $\square$

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Efficient photovoltaic devices for InP semiconductor/liquid junctions

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AN alternative to conventional solid-state photovoltaic devices is the semiconductor/liquid junction. Liquid-junction cells not only offer the possibility of integrating energy conversion and storage functions¹, but also may exhibit electrical properties that are fundamentally different from those in solid-state systems². We have investigated the photovoltaic behaviour of n-InP/metal and n-InP/liquid junctions. We have found that the electrical properties of these semiconductor/liquid junctions are superior to those of n-InP/metal (Schottky barrier) systems, and that the current-voltage characteristics are a strong function of the electrochemical potential of the liquid phase. Liquid contacts thus provide a possibility for the construction of more efficient photovoltaic devices than those available at present from Schottky barriers.

The n-InP samples were 300- μm -thick, $2 \times 10^{16} \text{ cm}^{-3}$ Si-doped crystals oriented along the (100) plane obtained from Crystacomm Inc., Mountain View, California. After mounting³ and etching⁴, the samples were immersed in the appropriate electrolyte solution and current-voltage (I - V) data were collected. The redox potential $E(A^+/A)$ of the $\text{LiClO}_4\text{-CH}_3\text{OH}$ electrolyte was varied by use of several different pairs of oxidized (A^+) and reduced (A) ions. Because of their different electron affinities and solvation properties, addition of these redox pairs (A^+/A) to the liquid produced changes in the electrochemical potential (that is, the Fermi level) of the solution phase. On the electrochemical potential scale, more positive redox potentials indicate that the Fermi level of the solution is further away from the vacuum level. Thus, the difference in energy between the conduction band of an n-type semiconductor and the redox potential of the solution (that is, the barrier height) should be larger for more positive solution redox potentials. The molecules used included: cobaltocene^{+/0} (with a standard electrochemical potential $E^0 = -0.9 \text{ V}$ relative to the reference level of a standard calomel electrode (SCE) (CoCp_2); decamethylferrocene^{+/0} ($E^0 = -0.08 \text{ V}$ relative to SCE) (Me_{10}Fc); 1,1'-dimethylferrocene ($E^0 = +0.26$ relative to SCE) (Me_2Fc).

Figure 1 displays the dark I - V properties of n-InP/Au Schottky contacts and n-InP/0.20M Me_2Fc -0.10M Me_2Fc^+ -1.0M $\text{LiClO}_4\text{-CH}_3\text{OH}$ junctions. In accord with previous reports, we observed that the Schottky contacts exhibited ohmic behaviour at moderate current densities and were poor rectifying contacts with high reverse-saturation-current (J_0) values^{5–9}. By contrast, the dark I - V properties of the dimethylferrocene- CH_3OH liquid contact had excellent rectifying characteristics and low J_0 values ($J_0 = 10^{-9} \text{ A cm}^{-2}$).

This difference in rectification implies that more efficient photoelectrochemical behaviour should be obtainable from the n-InP/liquid interface. Figure 2 displays the potentiostatic photoelectrode I - V properties of the n-InP/0.20M Me_2Fc -0.5mM Me_2Fc^+ -1.0M $\text{LiClO}_4\text{-CH}_3\text{OH}$ junction under simulated Air Mass 2 conditions (62 mW cm^{-2}) and under lower-level illumination. At Air Mass 2 conditions, we observed short-circuit current densities of $14\text{--}15 \text{ mA cm}^{-2}$, open-circuit voltages (V_{oc}) of $0.59\text{--}0.61 \text{ V}$ and photoelectrode efficiencies of $6.5\text{--}7.0\%$, which are quite excellent properties for an n-InP surface-barrier device. The modest fill factors (rectangularity of the I - V curves¹⁰) arise from residual concentration overpotentials and uncompensated series-resistance losses in the test-cell design¹¹, both of which can be minimized by optimal electrode-electrolyte configurations¹². The I - V properties at lower light levels ($<10 \text{ mW cm}^{-2}$) show (Fig. 2b) that improved fill factors are obtained when

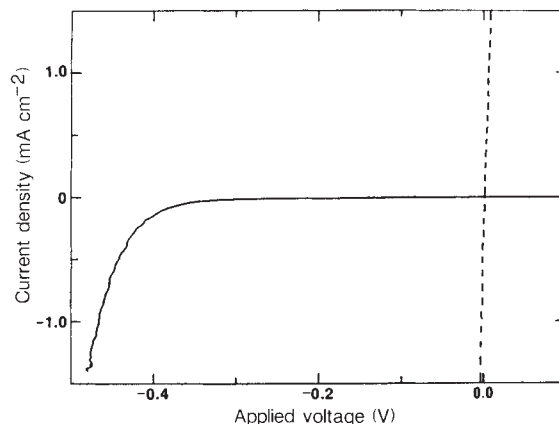


FIG. 1. Dark I - V properties of the n-InP/ CH_3OH -0.20M Me_2Fc -0.10M Me_2Fc^+ semiconductor/liquid junction (solid line) and of the n-InP/Au Schottky barrier (dashed line). Negative voltages are forward biases and anodic currents are positive. n-InP Schottky barriers were made by filament evaporation of the metal in a vacuum of base pressure $<5 \times 10^{-6}$ torr.

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these losses are minimized. Photoelectrode stability was confirmed for charge passed $>10^2 \text{ C cm}^{-2}$, which is in agreement with previous rotating ring-disk studies on a related n-InP/CH₃OH interface¹³ and indicates that the observed photovoltages are not an artefact of a corrosion potential acting in series with the regenerative cell photovoltage.

The I - V properties of the n-InP/CH₃OH junction were also found to be sensitive to the redox potential of the electrolyte. Figure 3 depicts the I - V behaviour of n-InP/Me₁₀Fc^{+/0}-LiClO₄-CH₃OH-tetrahydrofuran and n-InP/CoCp₂^{+/0}-LiClO₄-CH₃OH junctions. The CoCp₂^{+/0} system has the most negative redox potential (that is, the Fermi level that is closest to the vacuum level), and this solution produced poor rectification properties with n-InP samples. The n-InP/Me₁₀Fc^{+/0} system had higher V_{oc} values and better dark-rectification properties, in accord with expectations for an unpinned semiconductor surface Fermi level over this range of solution redox potentials. This behaviour is consistent with

dark-current cyclic-voltammetry studies of related n-InP/CH₃CN interfaces¹⁴ but contrasts with the low barrier heights found for various n-InP/metal contacts⁵⁻⁹.

The three metallocenes used in this study are sufficiently similar in their charge-carrier capture properties (that is, their solvent reorganization energies and heterogeneous electron-capture rates)¹⁵⁻¹⁸ that the most reasonable explanation for the differences between Figs 2 and 3 is a large change in the barrier height of the n-InP/liquid contacts with changes in the value of the solution redox potential. The changes in the reverse saturation current are in good agreement with expectations based on increasing barrier heights with more positive solution redox potentials. Furthermore, the data for the lowest J_0 system, n-InP/CH₃OH-Me₂Fc^{+/0}, cannot readily be rationalized by assuming that the barrier height of the semiconductor/liquid contact is pinned at the 0.3-0.5 V value of n-InP semiconductor/metal junctions. Using a 0.4 V barrier height in the thermionic-emission expression^{5-9,19}, we calculate that $J_0 = 0.16 \text{ A cm}^{-2}$ for the Schottky contact; thus, J_0 for the liquid contact would have to be decreased by over eight orders of magnitude to yield the observed dark I - V properties of Fig. 1 and to produce the V_{oc} values depicted in Fig. 2.

To explain the very low value of J_0 for the n-InP/Me₂Fc^{+/0}

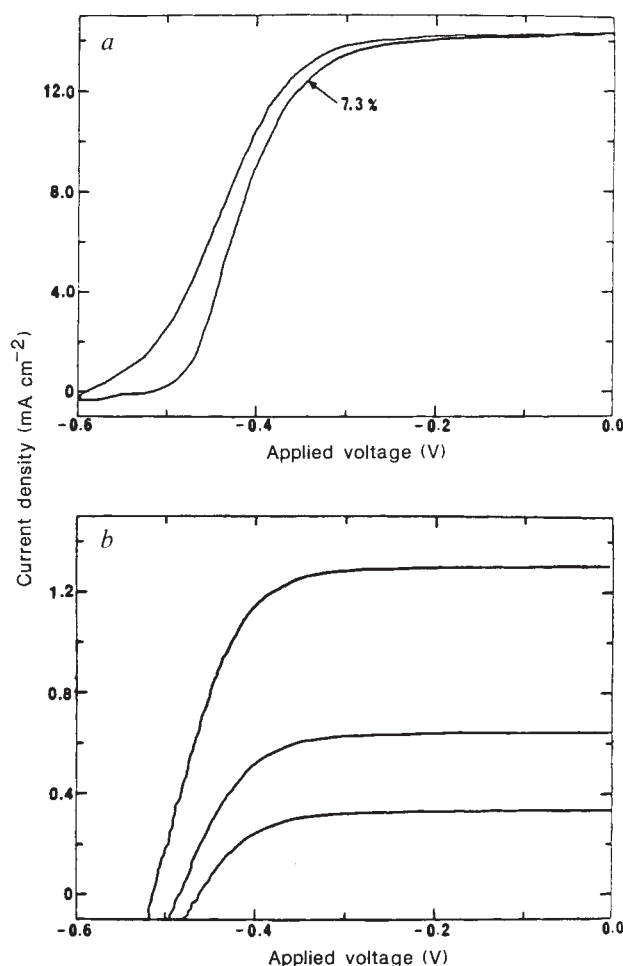


FIG. 2 *a*, I - V properties of the n-InP/CH₃OH-1.0M LiClO₄-0.20M Me₂Fc-0.5mM Me₂Fc⁺ junction under Air Mass 2 conditions (62 mW cm^{-2}). The electrode was mirror-finished (100)-oriented n-InP and the solution was magnetically stirred. The CH₃OH was distilled from Mg and stored over 3-Å molecular sieves. The LiClO₄ was fused *in vacuo* at 280 °C. Illumination was provided by an ELH-type 3,200-K, 100-W tungsten-halogen lamp³. The photoelectrode properties were obtained under potentiostatic control relative to a reference electrode at the Nernstian potential of the cell ($E = 0.15 \text{ V}$ relative to SCE). The hysteresis between forward and reverse direction scans was largely due to mass-transport limitations of the ions in the solution. The energy conversion efficiency of this particular interface was 7.3%. *b*, I - V properties at lower photocurrent densities showing the improved I - V behaviour at lower concentration overpotentials and series-resistance losses.

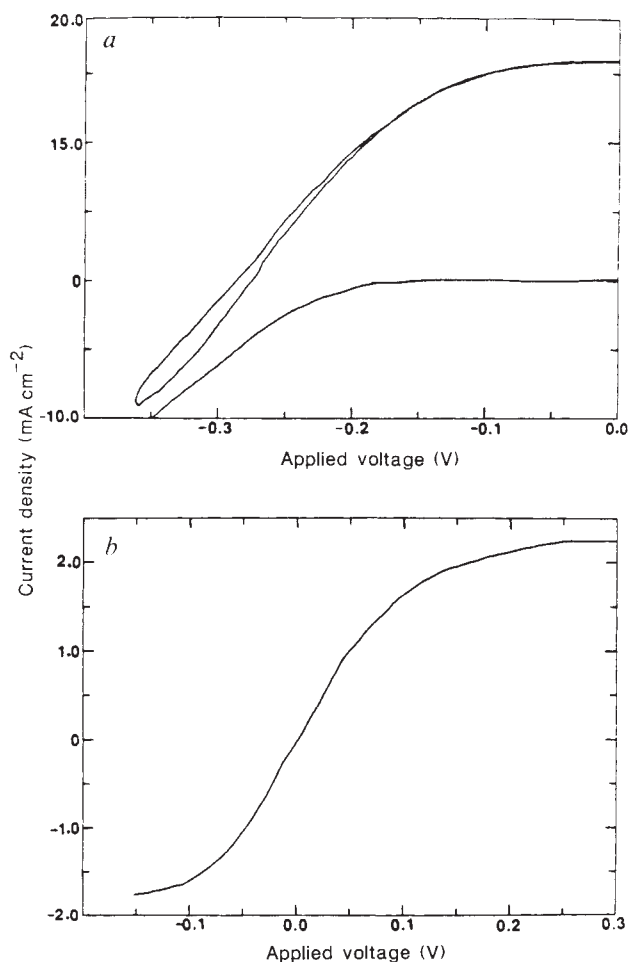


FIG. 3 *a*, I - V properties of the n-InP/CH₃OH (75%)-tetrahydrofuran (25% by volume)-0.50M LiClO₄-40.0mM Me₁₀Fc-0.5mM Me₁₀Fc⁺ system ($E = -0.107 \text{ V}$ relative to SCE) in the dark (lower curve) and under illumination. (Note the scale change on the abscissa as compared to Figs 1 and 2.) *b*, I - V properties of the n-InP/CH₃OH-1.0M LiClO₄-20.0mM CoCp₂-1mM CoCp₂⁺ ($E = -1.10$ relative to SCE) interface in the dark. Compared to the I - V data in *a* and Fig. 1, this system shows much higher dark currents and poorer rectification. I - V curves under Air Mass 2 illumination exhibited very low ($<10 \text{ mV}$) V_{oc} values.

system, it is necessary to postulate either a large tunnelling barrier at the n-InP/CH₃OH interface, an anomalously low cross section of electron capture for the Me₂Fc⁺ ion or a substantially increased barrier height for the liquid junction. The presence of a thick, insulating tunnelling barrier is ruled out by the low series resistance for charge transfer evident in the *I*-*V* data of Figs 1 and 2. Also, X-ray photoelectron spectra indicate that n-InP photoelectrode surfaces that have been operated in the CH₃OH-Me₂Fc^{+/-0} electrolyte possess ≤ 1.2 Å of oxide overlayers, which is consistent with the low levels of oxide observed by other workers after emersion of InP from other liquids²⁰. Postulating a low electronic cross section of carrier capture for the Me₂Fc⁺ ion would be in direct conflict with the excellent dark *I*-*V* behaviour in Fig. 1 and is also in contradiction to the rapid, reversible electron transfer rates exhibited by ferricenium ions at numerous electrode surfaces¹⁵⁻¹⁷. For liquids, some suppression of electron injection rates might be expected because of the necessity for solvent reorganization around the acceptor ion and the relatively low concentration of acceptor states²¹. A discrepancy of 10^8 in exchange current is, however, far too large to be explained in this fashion²¹. We thus conclude that the effective barrier height for majority-carrier transport in the n-InP/Me₂Fc^{+/-0}-CH₃OH semiconductor/liquid system is much larger than that obtainable for n-InP semiconductor/metal Schottky barriers.

Additional information regarding the recombination processes at the n-InP/CH₃OH interface can be obtained² from plots of *V*_{oc} against temperature *T*. Such plots for the n-InP/CH₃OH-Me₂Fc^{+/-0} system (220-300 K) were extremely linear (correlation coefficient >0.995) and yielded an extrapolated intercept (*T*=0 K) of 1.2 V. This value is much higher than the 0.4-V intercept expected for electron exchange processes over a 0.4-V barrier height^{10,19}. Also, at low temperature (234 K) and higher light intensity (~300 mW cm⁻² of ELH-type W-halogen illumination), we observed *V*_{oc} values >0.81 V. These data, combined with the room temperature *I*-*V* data of Fig. 1, clearly indicate that restrictions on photovoltaic performance and on junction leakage predicted by Fermi-level pinning at Schottky barriers do not apply to n-InP/CH₃OH liquid interfaces. It is this difference between the interfacial properties of liquid/semiconductor and metal/semiconductor junctions that enables the construction of efficient photoelectrochemical cells from semiconductors such as n-GaAs and n-InP, even though these materials have been shown to yield inefficient Schottky-barrier photovoltaic devices.

The n-InP system provides a key comparison in the III-V family of semiconductors because it is well documented that the surface Fermi level is pinned very near (0.3-0.5 V) the conduction band for a variety of metal contacts⁵⁻⁹. This implies that extremely poor InP junction behaviour should be obtained for all such systems. Previous reports have observed inferior *I*-*V* properties of n-InP/liquid contacts²²⁻²⁴ and differential capacitance measurements have been interpreted as indicating a high density of surface states pinning the Fermi level of n-InP/liquid junctions²⁵. By contrast, our data on n-InP/liquid systems clearly show that excellent photoelectrochemical-cell systems can be obtained despite the limitations of the solid-state devices, and that the *I*-*V* behaviour of the semiconductor/liquid devices can be extremely responsive to the electrochemical potential of the contacting phase. In fact, the *I*-*V* properties of the mirror-finished, (100) n-InP/CH₃OH-Me₂Fc^{+/-0} semiconductor/liquid junctions in Fig. 2 are comparable to those obtained from mirror-finished, (100) n-GaAs interfaces²⁶, which have yielded photoelectrochemical-cell efficiencies of >10% after optimization of surface etching and electrochemical-cell configurations^{2,26}. In conjunction with the recent studies of passivation of III-V surfaces at interfaces with non-conductors²⁷⁻²⁹, the observation that efficient energy conversion systems can be obtained with n-InP/liquid contacts demonstrates that semiconductor/metal barrier-height measurements are not a

reliable guide to the *I*-*V* behaviour of III-V semiconductor/liquid interfaces and that new options are available for the utilization of semiconductor/liquid systems in energy conversion applications. □

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Direct measurement of the diffusive sublayer at the deep sea floor using oxygen microelectrodes

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THE diffusive sublayer is the region of fluid next to a solid surface, where turbulence is suppressed and molecular diffusion dominates transport of solutes. Diffusive impedance of solute exchange across the benthic sublayer in the deep sea can limit the rates of some diagenetic reactions in the sediment. We present the first direct *in situ* measurements of the thickness of the diffusive sublayer for dissolved oxygen in the deep sea. The positions of 17 oxygen microelectrode profiles relative to the visible sediment/water interface reveal that the sublayer is 0.5-1.5 mm thick, with measurements ranging to 3.5 mm. The sublayer reduces the diffusive flux of oxygen into the sediments by ~10% in these environments. Also, the diffusive flux of isotopically light carbon through the sublayer should cause the ¹³C content at the interface of typical deep-sea sediments to be ~0.1% lighter than the bottom-water value, setting a limit on the precision of the record of past bottom-water given by the carbon isotope composition of the shells of benthic foraminifera.

Sedimentary diagenetic reactions that may be influenced by the diffusive sublayer include the dissolution of CaCO_3 (refs 1–3), the growth of manganese nodules⁴ and oxic respiration in sediments underlying regions of high productivity⁵. Also, it is important to know the thickness of the diffusive sublayer for interpretation of benthic fluxes measured directly using the flux-chamber method^{6,7}.

Measurements of the sublayer thickness for O_2 in a laboratory flume ranged from 1.1 mm for a bulk flow velocity of $1\text{--}2\text{ cm s}^{-1}$, to 0.5 mm for a velocity of $10\text{--}20\text{ cm s}^{-1}$ (ref. 5). The thickness of the diffusive sublayer above flat plates of dissolving alabaster placed on the ocean floor has been estimated to be $\sim 1.8\text{ mm}$ (ref. 8, see ref. 4 for reinterpretation). These measurements are in accord with hydrodynamic estimates⁹ ranging from 0.5 to 2.0 mm, for a friction velocity of the bottom water, U_* , in the range $0.05\text{--}0.3\text{ cm s}^{-1}$, which is typical of the ocean bottom¹⁰. The plate dissolution experiments and the hydrodynamic estimates both assume unstratified fluid flowing above a smooth flat wall, but the diffusive sublayer over deep-sea sediments could be affected by gradients in viscosity or density caused by

flocculent sediment above the interface (for example, see ref. 11) or by surface topography^{5,12}.

In October 1987 and November 1988 we measured the thickness of the sublayer for O_2 *in situ* by positioning a microelectrode profiling system³ at the sediment surface in the Santa Catalina Basin^{13,14} using the submersible research vessel *Alvin*. The profiler was also deployed outside a remote benthic tripod on the shelf and slope of Washington State¹⁵ in June 1988. In all locations the concentration of dissolved oxygen decreased from bottom-water values to unmeasurable levels within 4–8 mm (Fig. 1). We estimate the depth at which the electrode crossed the sediment/water interface by mounting 2-mm diameter dowels, painted with high-contrast white and black bands, next to each electrode and monitoring, by video recording, the penetration of the dowels into the sediment (Fig. 2). The thickness of the sublayer can be estimated with a precision of the $\sim 0.5\text{-mm}$ vertical spacing of the oxygen measurements. A fibre-optic transmissivity sensor was installed for some of the deployments (June and November 1988), which enabled us to measure bottom-water turbidity as close as 1 mm above the bed.

We determined the decimetre-scale topography at each deployment site by using the relative depths at which each of the four electrode tips penetrated the sediment. If the surface slope of the site exceeded a few per cent, and the measuring stick was positioned uphill or downhill from its electrode, the sublayer thickness estimate may have been biased and we excluded it from consideration. In each of the rejected profiles, the sticks happened to be placed uphill of the electrodes, potentially underestimating the sublayer thickness. The remaining estimates are summarized in Fig. 3. The thickness of the sublayer is operationally defined as the vertical distance between the lowest depth at which bottom-water O_2 was detected and the location of the visible sediment/water interface.

The distribution of sublayer thickness measurements from each region varied by $\pm 0.5\text{ mm}$. Much of this variation occurs in profiles $\sim 10\text{ cm}$ apart, from the same deployment. The reproducibility of the measurement at each location to within the uncertainty of the technique implies that sediment topography on the scale of the distance from the stick to the electrode tip ($\sim 1\text{ cm}$) was not large enough to affect our results significantly. An attempt to interpret the $\pm 0.5\text{-mm}$ variation in terms of environmental parameters would, however, probably be misleading.

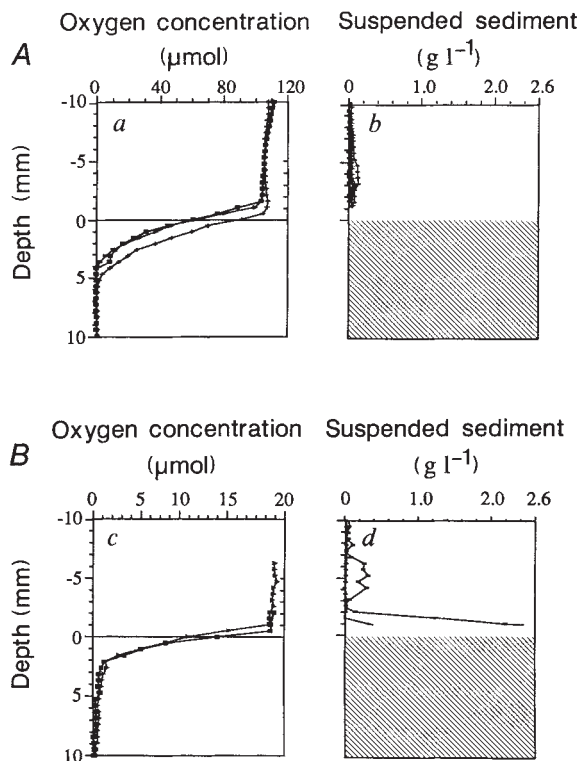


FIG. 1 Sediment/water interface oxygen concentrations and bottom-water transmissivity profiles measured *in situ*. A on the shelf and slope of Washington State and B in the Santa Catalina Basin. a (top and bottom), Representative oxygen profiles from one deployment in each region. The oxygen microelectrodes were similar to those reported in ref. 22. Their sensitive tips are 0.05 mm in diameter, and their response times are $\sim 1\text{ min}$. The electrodes were linear at oxygen tensions of $2\text{--}150\text{ }\mu\text{mol l}^{-1}$ and the output decreased $<2\%$ in response to the transition from vigorous stirring to stagnant conditions (see ref. 3 for further discussion), a much smaller change than was observed across the diffusive sublayer. b (top and bottom), All the near-bed turbidity data from multiple deployments in each region. The transmissivity sensor consisted of two fibre-optic cables, 1 mm in diameter, facing each other, 8 mm apart. The extinction coefficient for suspended surface sediment was measured in the laboratory. The sensor has a detection limit of 0.1 g l^{-1} or better of suspended sediment *in situ*. No increase in near-bed turbidity was detected over the sandier sediments of the Washington Shelf (b, top). Turbidity values found above sediments in the Santa Catalina Basin (b, bottom) are three orders of magnitude greater than reported values in the nepheloid layer hundreds of metres away from the bed in the California Borderlands²³.

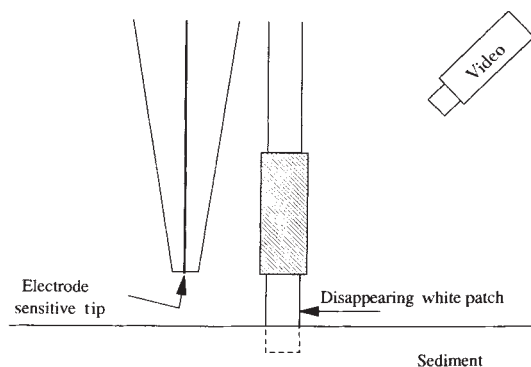


FIG. 2 The method of finding the sediment/water interface for the oxygen profiles. The spatial relationship between the white-dark boundary of each rod and its corresponding O_2 electrode tip was used to locate the visible sediment/water interface, independently for each electrode, using video recording. The camera was placed at an angle of 50° from the vertical. The light was focused by a lens of focal length 8.5 mm onto a charged-coupled sensor that was $8.8 \times 6.6\text{ mm}$. The vertical resolution of the recorded was $\sim 0.25\text{ mm}$ per pixel with the camera placement used. Laboratory tests confirmed that the video camera was able to resolve the disappearance of the tip to $<0.5\text{ mm}$. In sediments with a flat interface, positioning the stick at a horizontal distance of 1 cm from the electrode gave, on average, the same results as using a distance of 0.3 cm , suggesting that disruption of the bottom current by the stick did not significantly bias the results.

There does seem to be a systematic difference between the results from the single deployment at Santa Catalina Basin in October 1987 and the other observations, including data from the same location one year later (Fig. 3). We have detected no experimental flaws in this deployment. If the thicker sublayer were caused by lower bottom-water turbulence, the flow velocity would have to be roughly five times lower than in the other observations⁹, which was not supported by the drift rates of suspended particulates in the video records or by visual observations from *Alvin*. The observations could also be explained by the presence of a 1- to 2-mm-thick layer of flocculent material at the interface. No such layer was visible from *Alvin*, however, and the turbidity sensor was not deployed at this time, so this hypothesis cannot be verified.

We observed a near-bed increase in turbidity in the Santa Catalina Basin one year later, in contrast to results from the sandier sediments on the Washington shelf (Fig. 1). In spite of the difference in suspended particulate loads, the diffusive-sublayer thickness data from the two locations are probably not significantly different. If the solid material in the turbidity layer was not attached to the bed in any way, it should have increased the suspension viscosity by $\sim 2\%$ g l^{-1} of suspended sediment¹⁶. The diffusive-sublayer thickness is expected to increase proportionally (1–5% total change for the turbidity observed)⁹.

Except for the first Santa Catalina Basin cruise results, the diffusive sublayer is ~ 1 mm thick, in accord with hydrodynamic prediction. Although the precision of these measurements is not as great as obtained by the plate-dissolution method⁸, this study shows that the assumption of unstratified fluid flowing over a smooth, flat wall is not fundamentally in error for the sublayer over fine-grained deep-sea sediments. This assumption is integral to sublayer thickness estimates based on the dissolution experiments and on hydrodynamic theory⁹.

Diffusive-sublayer limitation of solute-controlled sediment reaction rates is most important if the characteristic reaction time of the solute in the pore water is shorter than the solute transport time across the sublayer. Thus, the sublayer exerts greater control of the sedimentary oxygen flux as the penetration depth of oxygen into the sediment decreases, namely, in areas of high carbon rain or low bottom-water oxygen concentration. Our calculations indicate that, for O_2 -penetration depths typical of this data set, a diffusive sublayer 1 mm thick decreases the oxygen flux by $\sim 10\%$ relative to the case of no sublayer (see Fig. 4). Limitation of the oxygen flux into the sediments would

act to enhance the preservation of organic carbon in these environments, if oxidic respiration is more effective than anaerobic metabolism¹⁷.

The sublayer gradient in the carbon-isotope ratio of dissolved inorganic carbon (DIC) due to the flux of isotopically light metabolic CO_2 may pose limits on the precision of benthic-foraminifera tests as recorders of past bottom-water ^{13}C content (Fig. 4). For organic carbon rain rates typical of the pelagic sediments where palaeoceanographic foraminifera samples are usually taken ($10\text{--}50 \mu\text{mol C cm}^{-2} \text{ yr}^{-1}$ (ref. 3)), there should be a 0.1–0.3‰ transition in the sublayer between the $\delta^{13}\text{C}$ DIC value in bottom water and at the interface. Because benthic foraminifera are small enough to live within the sublayer¹⁸, they could record the $\delta^{13}\text{C}$ DIC of this region. Some foraminifera species have been observed to position themselves outside the sublayer on stalks¹⁹; unless the entire population does this

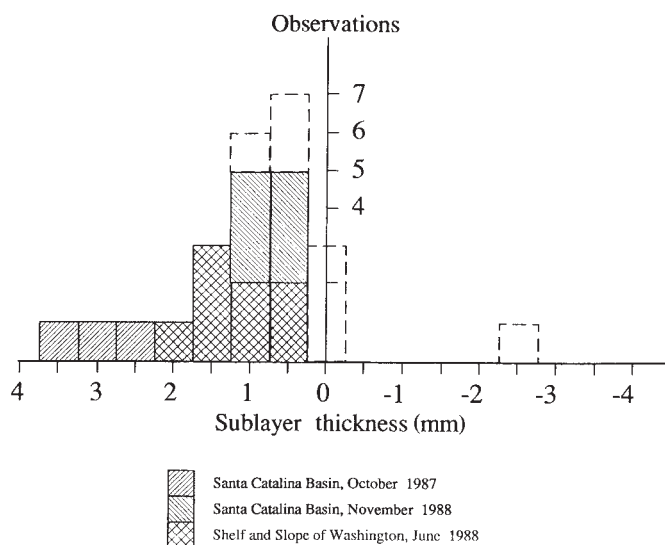


FIG. 3 Summary of 17 *in situ* determinations of the thickness of the diffusive sublayer. The observations indicated by dashed lines are probably an artefact of surface topography (see text). The study confirms that the thickness of the diffusive sublayer is usually 0.5–1.5 mm.

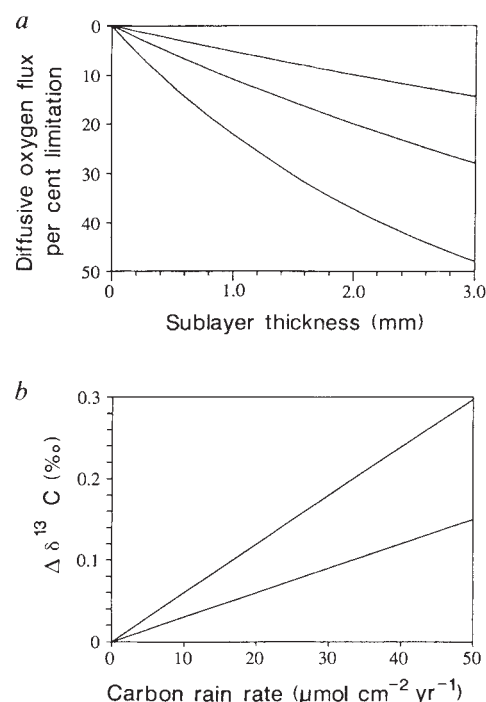


FIG. 4 (a), The response of the diffusive oxygen flux into the sediments to thickness of the diffusive sublayer for O_2 penetration depths (with no sublayer) of 2.5, 5.0 and 10.0 mm (lower, middle and upper curves, respectively). As the sublayer thickness increases oxygen penetrates less deeply into the sediments and there is less organic carbon within the oxic zone available for reaction, reducing the oxygen flux into the sediments. The steady-state profile of oxygen is governed by the processes of diffusion and reaction^{3,24}. In the sediment, the reaction rate is modelled as first-order with respect to carbon, and zero-order with O_2 , until O_2 is depleted. The reaction rate within the sublayer is zero. The organic carbon concentration with depth is assumed to be proportional to the volume fraction of solids in the sediment (the complement of the porosity profile, derived from measured resistivity data and fit to an exponential^{3,25}). Assuming no sublayer, and specifying the penetration depth of O_2 into the sediment, the kinetic rate constant for organic degradation is determined by equating the diffusive flux of oxygen into the sediment with the integrated degradation rate of the organic material within the oxic zone. The rate constant is held fixed as the sublayer thickness is increased to predict the effect of the sublayer on the benthic O_2 consumption rate. The results presented do not include the effect of the porosity on the diffusion coefficient, the effect of a potentially varying solid fraction of labile organic carbon with depth, or the possibility of O_2 reacting with dissolved species. b, The difference between the $\delta^{13}\text{C}$ of DIC at the sediment/water interface and in the bottom water, $\Delta\delta^{13}\text{C}$, plotted as a function of the particulate-organic-carbon rain rate for diffusive-sublayer thicknesses of 0.5 and 1.0 mm (lower and upper curves, respectively). Gradients in $\delta^{13}\text{C}$ expected within the sublayer were calculated by extrapolating pore-water $\delta^{13}\text{C}$ data^{26,27} to the sediment/water interface.

uniformly, however, the gradient of $\delta^{13}\text{C}$ DIC within the diffusive sublayer could easily result in the 0.1‰ variation in the carbon isotope ratio observed^{20,21}. □

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Distortions, rotations and crustal thinning at ridge–transform intersections

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WHEN an oceanic ridge intersects a transform fault, the newly formed oceanic crust may be severely distorted, frequently exhibiting major rotations of fault blocks^{1–4}. Such effects typically extend over a 10-km-wide strip parallel to the transform. By contrast, studies of contemporary oceanic transforms suggest that deformation is restricted to a single fault trace within the transform domain^{5–9}. This apparent inconsistency can be reconciled if the rotations occurred at the ridge–transform intersection during accretion of the crust. Here I develop a kinematic model that considers the effect of an ephemeral period of stretching on a region that broadens as the transform is approached along the ridge. The strains calculated from this model indicate extension at the ridge, simple shear at the transform and composite strains in the region of distributed deformation. The model predicts very rapid rotation rates (100° in 10^4 – 10^5 yr). The implications for the topography and structure of ridge–transform intersections are considered, and the alternating periods of extension by stretching and by intrusion are illustrated by reference to contemporary transforms on the Mid-Atlantic Ridge. The model may also explain the common occurrence of cross-cutting dykes near transform faults in ophiolites.

Large clockwise rotations of a dextral dyke swarm have been observed to the south of the dextral-slipping Tjournes fracture zone in Iceland¹. Heterogeneous simple shear has affected a 10-km wide strip, with rotations locally apparently $>90^\circ$ (Fig. 1a). A similar effect is noted on the Troodos ophiolite of Cyprus to the north of the dextral-slip Arakapas transform fault, where clockwise fault-block rotations have been confirmed by palaeomagnetic studies^{2,3} (Fig. 1b). Again, the region affected is about 10 km wide. In the eastern part of the complex a ridge jump has preserved crust formed at the non-transform corner of the ridge–transform intersection³ that does not exhibit significant rotations about vertical axes. Large rotations and stretching lineations have been recorded from deeper structural levels at the Newfoundland coastal complex ophiolite⁴ (Fig. 1b), up to 5 km from a fossilized oceanic transform fault. In these three examples where transform faults are exposed in ophiolites, the crustal sequence adjacent to the fault has been rotated, locally $>90^\circ$. In the examples from both Iceland and Cyprus, the rotated material is deformed by brittle faulting outside the transform domain, the domain being defined by distinctive escarpments in each case.

In contemporary oceanic transform faults, strike-slip deformation seems to be confined largely to the transform zone, and active tectonism is restricted to a very narrow belt less than 1 km wide^{5–9}. Although the width of the plate-bounding shear zone may increase with depth, it is not clear how this could develop the rotations in the oceanic crust of the transform walls.

An appealing alternative is that the rotations occurred very close to the plate boundary, at the ridge–transform intersection, when it was still hot and weak enough for brittle failure to occur. Recent studies of the structure of intermediate- to slow-spreading oceanic ridges suggest that during periods of reduced magma supply, in the absence of a magma chamber^{10–13}, extension is accommodated by normal faulting. Seismic reflection profiles from the fast-spreading East Pacific Rise¹⁴ show that a magma chamber is not in evidence next to the Clipperton transform. Magma chambers thus may frequently be absent at ridge–transform intersections and stretching may be a common phenomenon in this environment. This is supported by the microseismicity at ridge–transform intersections^{15,16}. Earthquakes are frequently scattered in the transform corner, rather than delineating a narrow plate boundary, which suggests that brittle deformation may be distributed over a broad region at the ridge–transform intersection.

My model explores some of the kinematic implications of a period of extension by faulting at a ridge–transform intersection, in terms of the induced strains and rotation rates. Two rigid plates at a ridge–transform intersection are defined and deformation is assumed to be restricted to the area between them. A simple two-dimensional velocity structure for points in the deforming region is assumed, such that

$$u = kxy - u_1/2 \quad (1)$$

$$v = 0 \quad (2)$$

(see Figs 2 and 3). Plate A moves at $u = -u_1/2$ and plate B, with a boundary defined by $u_1 = kxy$, moves at $u_1/2$. This velocity structure was chosen as the simplest that gave $u = -u_1/2$ on both $x = 0$ and $y = 0$ and that gave an area of distributed deformation that broadened as the transform was approached.

This simple model illustrates the effects of a short-lived stretching event. At the ridge away from the transform (at the top of Fig. 2c) deformation is essentially pure shear producing vertical plane strain. Along the transform, simple shear develops horizontal plane strain. The strains are transitional between these end conditions. Strains with axial ratios of 1:2:10, similar to those described at the Newfoundland coastal complex, were generated after only 100,000 years of stretching across a 2-km zone⁴. Styles of brittle deformation on the Troodos ophiolite

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are also consistent with the strains developed here, with subsidiary strike-slip faults largely confined to the transform corner, and normal faults dominating in the non-transform corner³.

The rotations of strain ellipsoids are equivalent to those of passive markers. Using the velocity gradients, the rotation rate can be deduced for rigid particles within the flow. For many continental situations this rate is also appropriate for fault-blocks at the surface¹⁷.

The velocity vector $\mathbf{v}$ can be defined such that

$$\mathbf{v} = (u, v, w) = (kxy - u_1/2, 0, w) \quad (3)$$

so that the rate of rotation, or vorticity, ω , is given by

$$2\omega = \nabla \times \mathbf{v} = \left(-\frac{\partial w}{\partial y}, \frac{\partial w}{\partial x}, kx \right) \quad (4)$$

Thus $kx/2$ represents the instantaneous rotation rate about a vertical axis (ω_z). This implies that ω_z is proportional to the width of the deforming zone parallel to the transform, and is constant at any point along the transform. The modification of ω_z with time depends on how the boundaries of the deforming

zone are considered to evolve. Using realistic dimensions (a ridge 1–2 km wide and 10–20 km from the transform) and spreading rates ($5 \times 10^{-2} \text{ m yr}^{-1}$ – $10^{-1} \text{ m yr}^{-1}$), rotation rates of 10^{-4} – $10^{-5} \text{ rad yr}^{-1}$ can be generated, which would produce the ~ 2 -rad rotation observed at the Troodos ophiolite in 10^4 – 10^5 yr . Large rotations can occur within the dyke-intrusion zone, so that cross-cutting dykes should be common. These are observed close to fracture zones in ophiolites^{1,3,4}.

A period of amagmatic stretching requires lithospheric thinning, which will generate the deep nodal basin frequently associated with ridge-transform intersections. The rate of thinning is inversely proportional to the width of the deforming zone and so will increase with distance from the transform. This thinning will be balanced by material being injected laterally from a magma chamber some distance from the ridge. The deepest part of the nodal basin will thus be expected to lie between the

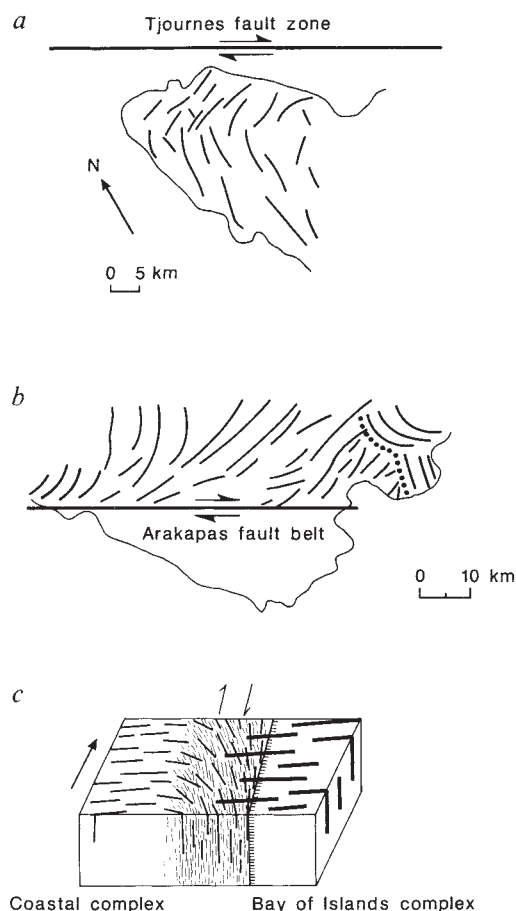


FIG. 1 Dyke trends at exposed fossil fracture zones. *a*, Tjournes fracture zone, north central Iceland¹. *b*, Arakapas fossil transform fault, Troodos ophiolite, Cyprus (east–west trend)^{3,22}. Note the major change in dyke strike to the east of the ridge-jump boundary (dotted line). Palaeomagnetic results^{2,3} indicate that dykes to the west of this boundary have been rotated by $\sim 100^\circ$ clockwise about steeply inclined axes, whereas those to the east have not. The area to the west is believed to have formed at the transform corner of a ridge-transform intersection, and those to the east, at the non-transform corner. *c*, Schematic diagram illustrating dyke trends on the Bay of Islands and coastal complex ophiolites in Newfoundland, in their preobduction setting⁴. Deformation of the coastal complex is by ductile shear. The coastal complex is believed to have formed at a transform corner, and the Bay of Islands at a non-transform corner, of a ridge-transform intersection.

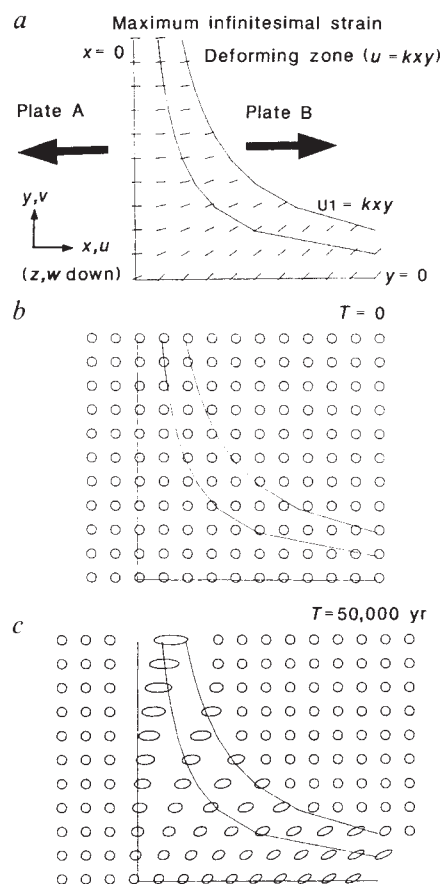


FIG. 2 Model for effect of stretching on the ridge-transform intersection. Displacement vector $\mathbf{x}_i$ is defined as parallel to the transform, $\mathbf{y}_j$ perpendicular to the transform and $\mathbf{z}_k$ vertical down. The corresponding velocity components are u, v, w . Plate A moves at $u = -u_1/2$, $v = 0$ and is defined by $y = 0$ and $x = 0$. Plate B moves at $u = u_1/2$ relative to plate A, and is defined by the boundary $u_1 = kxy$. The central line $u_1/2 = kxy$ remains fixed. The plates are considered rigid; deformation is restricted to the region between them and is described by the velocities $u = kxy - u_1/2$ and $v = 0$. *a*, Orientation of maximum infinitesimal strain axes. Dykes will be intruded perpendicular to these axes and thus will make a smaller acute angle to the transform at the transform corner than at the non-transform corner. This relationship is observed on the Troodos ophiolite³. *b*, Initial horizontal unit circles (radius = 1 m) and *c*, orientation after 50,000 years of stretching. Pure shear (extension perpendicular to the ridge) develops vertical plane strain at the ridge (top left), and simple shear (strike-slip) develops horizontal plane strain at the transform (bottom right). The strains are transitional between these end conditions. This is consistent with the orientation of brittle fractures on the Troodos ophiolite.

magma chamber and the transform fault. The cumulative thinning, however, is inversely proportional to the amount of new material injected, and will be greatest close to the transform, as is commonly observed on seismic refraction profiles^{16,18}. Such a thinning process would also explain the occurrence of lower crustal rocks frequently observed at the transform corner¹⁹. The anomalous uplift of the transform corner which has previously been attributed to serpentinized ultramafics would be enhanced by the increased faulting in the region predicted by this model.

Extension by stretching, illustrated in Fig. 3a, is exemplified by the eastern ridge-transform intersection of the Oceanographer fracture and the Mid-Atlantic Ridge²⁰, which is characterized by a deep nodal basin, largely devoid of sediment fill. The axis of accretion and the principal transform displacement zone are not clearly defined, and the neovolcanic zone is increasingly disrupted by fracturing as the transform is approached. The faults in the non-transform corner are ridge-parallel, whereas those at the transform corner have very variable orientations. Serpentinized ultramafics are exposed on the flanks of the basin.

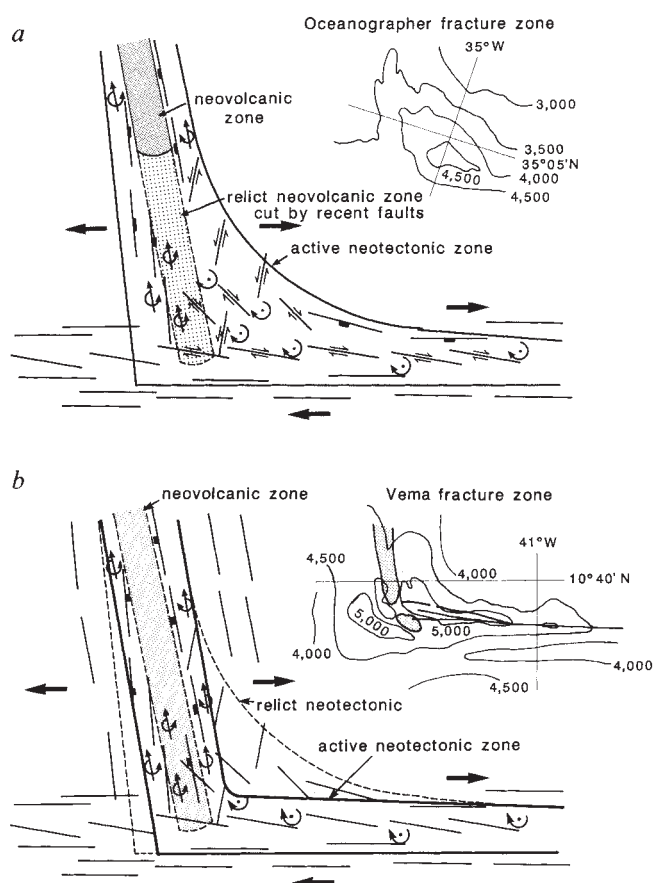


FIG. 3 *a*, Principal features of a ridge-transform intersection undergoing stretching, exemplified by the eastern intersection of the Oceanographer transform and the Mid-Atlantic Ridge (reversed for comparison)²⁰. A relict neovolcanic zone is truncated by active tectonism. The principal transform displacement zone is poorly defined within the well developed nodal basin. The model predicts the occurrence of normal faulting at the non-transform corner, and oblique-normal/strike activity at the transform corner. *b*, Extension by intrusion, exemplified by the eastern intersection of the Vema fracture zone with the Mid-Atlantic Ridge (rotated for comparison)²¹. Here, an active neovolcanic zone bisects a relict nodal basin, reflecting rejuvenated magmatic activity and uplift. The principal transform displacement zone is confined to a narrow, well defined trace. Tectonism is limited to a narrow region of normal faulting at the ridge, and of strike-slip faulting in the transform valley. (Depths in m.)

The following phase of rejuvenated neovolcanic activity, and a narrow deforming zone, can be identified in the eastern intersection of the Mid-Atlantic Ridge with the Vema fracture zone²¹ (Fig. 3b). Here, a volcanically but not tectonically active zone joins a well developed principal transform zone that is tectonically active, the various splays of which occupy a 2-km wide belt. The neovolcanic zone is elevated relative to the deep nodal basin that surrounds it.

The faults observed at ridge-transform intersections are dominantly dip-slip. This may reflect the dominance of vertical tectonism within this region, but does not preclude a small, but significant component of strike-slip movement, sufficient to produce the required rotation. This is the case for the eastern Greece continental graben system, where a rotation of up to 45° has been accommodated by a small component of strike-slip on otherwise normal faults¹⁷.

Ephemeral periods of stretching at ridge-transform intersections would generate the rotations observed in ophiolites. These rotations are probably accommodated by composite normal-strike slip faults in the brittle crust. Rotation rates may be high, and short periods of extreme tectonic activity may be expected. Cross-cutting dykes are a probable consequence of rotation close to the spreading axis. Between stretching events the plate boundaries will be narrower and more clearly defined by ridge and transform segments. Plate movement will be accommodated by dyke intrusion. □

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Accumulations of melt at the base of young oceanic crust

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AS hot, solid rock ascends through the mantle, the pressure around it decreases while its temperature decreases approximately adiabatically. During the ascent, the melting temperature decreases faster than the adiabat temperature, and if the initial temperature of the rock is high enough, the rock will begin to melt at the depth where the adiabat crosses the melting-temperature curve. Models of upwelling beneath mid-ocean ridges predict significant melt production over a zone several tens of kilometres wider than the ridge-axis region suggesting that some of the ascending melt must arrive away from the ridge axis. Here

I report ocean-bottom seismograph observations of an uncommon and previously unreported marine seismic phase, which provide strong evidence that melt accumulates in sills at the base of young oceanic crust. The phase results from the conversion of compressional (P) waves to shear (S) waves on interaction with the crust-mantle boundary (the Moho). This phase is rare and very difficult to model, but when visible, it is strikingly prominent and usually appears at unexpectedly great source-to-receiver ranges. The observations imply the presence of bodies of extremely low rigidity, presumably melt sills, at the Moho close to the receiver. Melt may be fairly mobile at the base of the crust, allowing ridges to accumulate melt through a lateral plumbing system at the locally uplifted boundary between the crust and the underlying thermally expanded mantle. Escape of this melt to the sea floor may be responsible for the formation of off-axis seamounts.

The phase due to a P wave in the crust reflected as an S wave from the Moho is designated¹ PmS, and includes lower-crustal-

diffracted P waves that are continuously converted to crustal S waves and which extend true PmS beyond its expected geometric range (~20 km). In addition, P waves refracted in the upper mantle and reflected from the underside of the Moho lose energy to S-wave radiation into the crust and mantle and also contribute to the apparent extension of the phase. I use the designation to denote all of these phases. The point of generation of the observed S waves of PmS is a horizontal distance under 4 km from the receiver in typical oceanic crust, even if the source is very distant. In a model with a simple sharp Moho, PmS is a very inconspicuous phase on the horizontal component of sea-floor displacement between the 15- and 20-km range and at a reduced time of ~3.9 s (Fig. 1). The synthetic seismograms show complete sea-floor motions assuming an overlying water half-space and isotropic elasticity. Phase-slowness integrations were

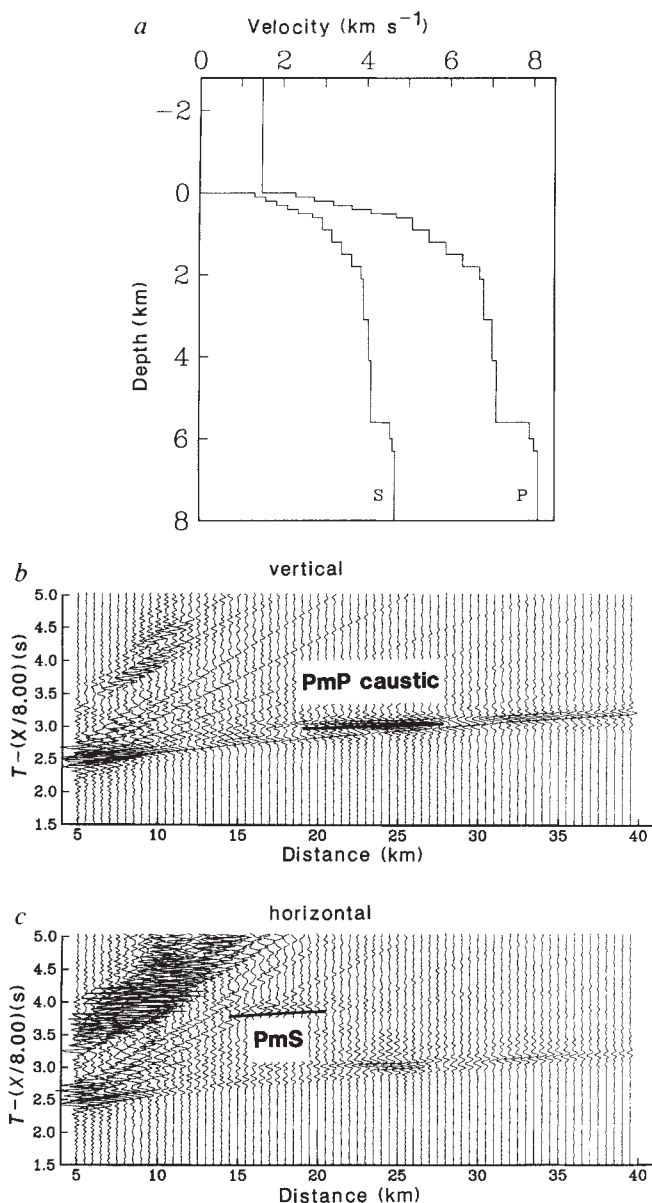


FIG. 1 *a*, Model and *b*, *c*, synthetic seismograms for simple Moho structure. Water depth 2.8 km. P and S velocities shown, densities linearly related to P velocity. PmS phase visible on horizontal (radial) component between 15- and 20-km range at a reduced time of 3.9–4.0 s. The reduced time is $T - (X/8.0)$ where T is the uncorrected time, X is the distance and 8 km s^{-1} is the reducing velocity.

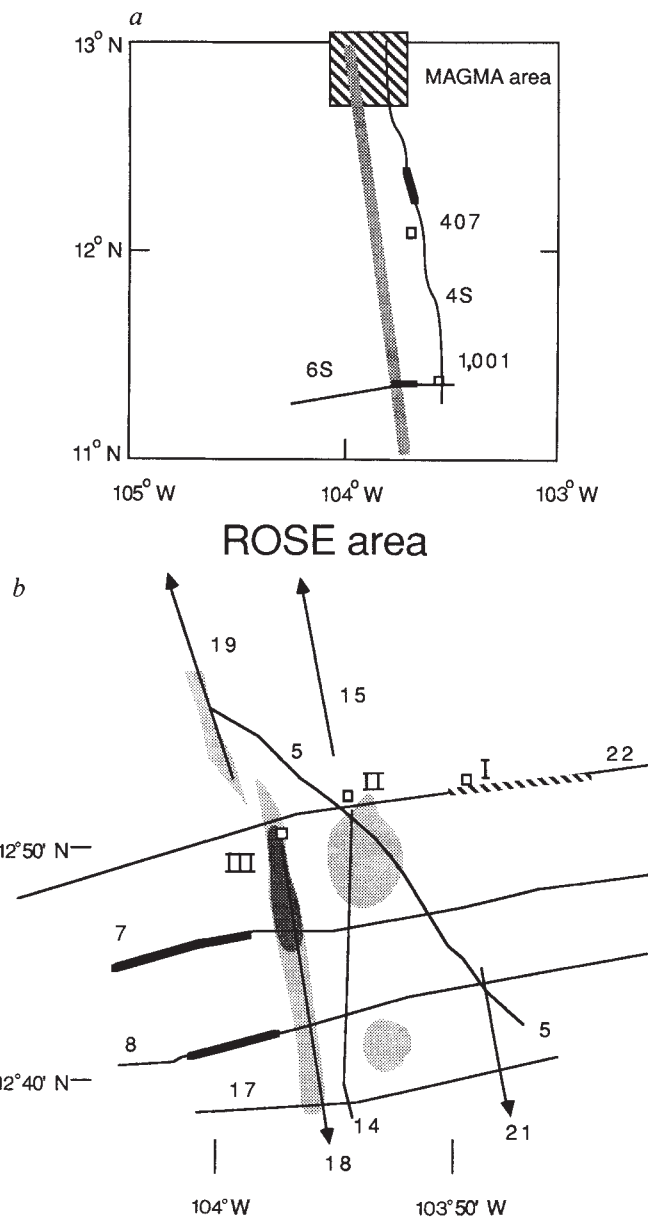


FIG. 2 Simplified location maps. *a*, ROSE area. Grey line is East Pacific Rise. OBS sites are open squares identified by site numbers. Shot lines are numbered, bold segments show the shots that yielded PmS. Hatched square shows approximate coverage of *b*. *b*, MAGMA area. Generally as in *a*. Grey areas are bathymetric highs (East Pacific Rise and off-axis seamounts). Darker grey shows location of known vent activity. Bold lines show shot locations of PmS observations at site II (solid lines) and disturbed propagation to site III (hatched line). Observations of PmS consistent with anomalous Moho between ridge and northern seamount.

calculated from 0.05 to 0.30 s km⁻¹ (3.33–20 km s⁻¹ phase velocity). Models that have transitional Moho structures that are thicker than a shear-wave half wavelength (~200 m for the middle of the frequency band used here, 5–15 Hz) yield much smaller PmS phases.

A practical difficulty in the observation of PmS is the presence of interfering P-to-S conversions of upgoing P waves just beneath the receiver. These shallow conversions are called Ps (ref. 1), where the s normally signifies that the S part of the path is in the sediments. The Ps waves in the data presented below are from nearly unsedimented regions², so the usage is somewhat loose—the conversion actually takes place at the base of the fractured basalt (layer 2a). The Ps phase is seen ~0.2–0.6 s after P; this wide range of delays is largely due to shear-wave anisotropy in the fractured basalt. Although these times are well before the arrival time of PmS (~1.2 s after P), the coda of Ps could make the identification of PmS difficult.

From the simple Moho model, it might be expected that PmS would almost never be observed because it is a weak phase which should be lost in the codas of the P and Ps arrivals. The horizontal-component ocean-bottom seismograph (OBS) data that I have examined largely confirm this conclusion. There are, however, some striking exceptions. A very strong PmS phase is visible on a horizontal (and nearly radial) component of an OBS in the Rivera Ocean Seismic Experiment (ROSE) experiment^{3,4} (MIT site 1,001, line 6S). Figure 2a shows a simplified location map of the ROSE area. The PmS phase is clearly distinct from the Ps phase and has quite high amplitudes beyond 20 km (Fig. 3a). The spatial distribution and sharp onset of the PmS phase rule out the coincidental reinforcement of S wave reverberations to give a false PmS. It is the appearance of PmS well beyond its expected range that argues most strongly that the Moho close to this instrument is unusual. The orthogonal line (4S) to this instrument shows no such phase, indicating that the

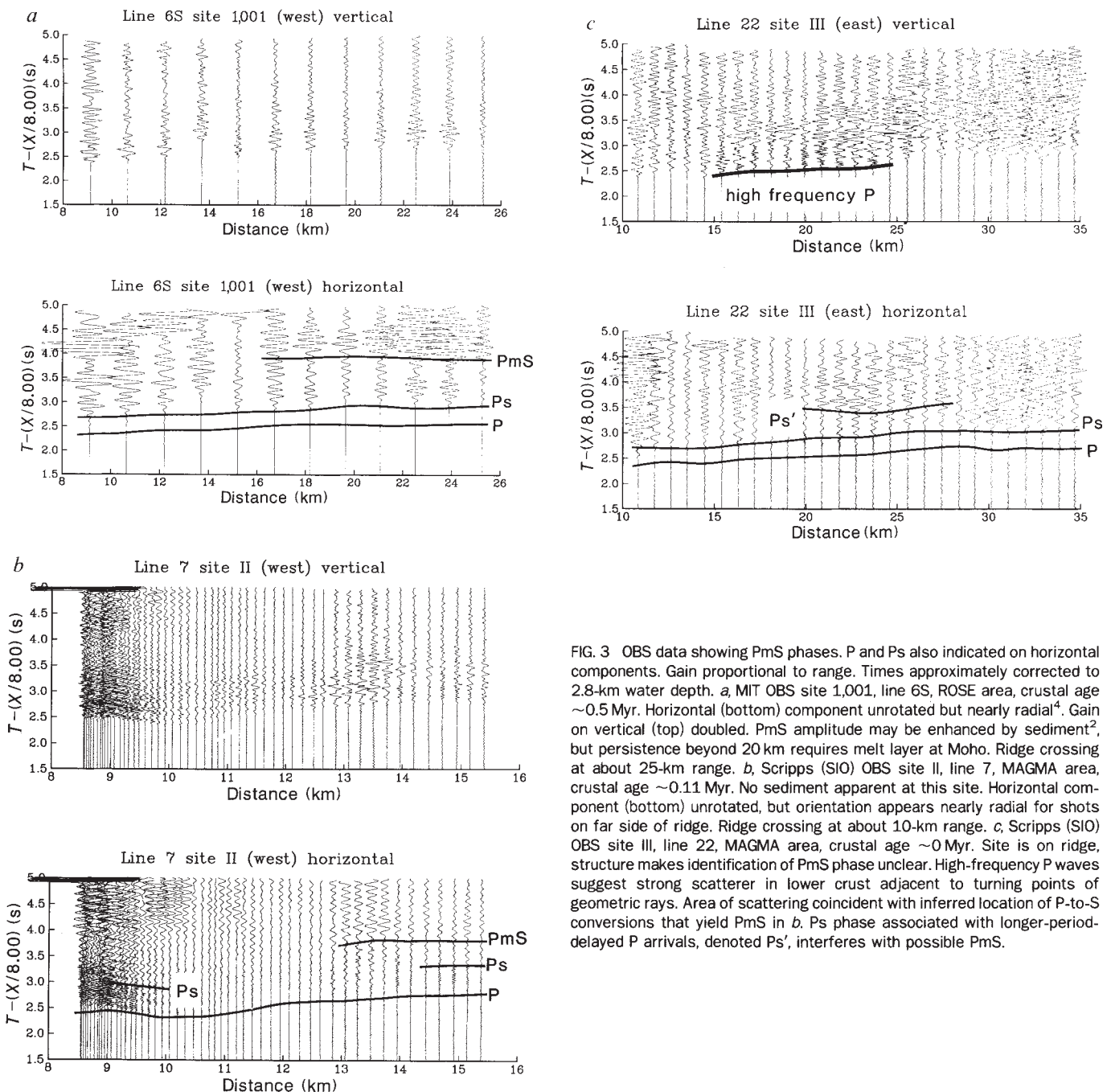


FIG. 3 OBS data showing PmS phases. P and Ps also indicated on horizontal components. Gain proportional to range. Times approximately corrected to 2.8-km water depth. *a*, MIT OBS site 1,001, line 6S, ROSE area, crustal age ~0.5 Myr. Horizontal (bottom) component unrotated but nearly radial⁴. Gain on vertical (top) doubled. PmS amplitude may be enhanced by sediment², but persistence beyond 20 km requires melt layer at Moho. Ridge crossing at about 25-km range. *b*, Scripps (SIO) OBS site II, line 7, MAGMA area, crustal age ~0.11 Myr. No sediment apparent at this site. Horizontal component (bottom) unrotated, but orientation appears nearly radial for shots on far side of ridge. Ridge crossing at about 10-km range. *c*, Scripps (SIO) OBS site III, line 22, MAGMA area, crustal age ~0 Myr. Site is on ridge, structure makes identification of PmS phase unclear. High-frequency P waves suggest strong scatterer in lower crust adjacent to turning points of geometric rays. Area of scattering coincident with inferred location of P-to-S conversions that yield PmS in *b*. Ps phase associated with longer-period-delayed P arrivals, denoted Ps', interferes with possible PmS.

conditions at the Moho that make PmS so visible on line 6S are spatially limited. The point of P-to-S conversion is ~ 22 km from the ridge.

Another instrument in the ROSE area (University of Washington site 407, line 4S north of the instrument) also shows an intense PmS arrival on its horizontal components in the range 20–28 km. The data show no evidence for PmS to the south of the instrument. Here, the point of P-to-S conversion is ~ 17 km from the ridge.

I have also examined data from the 1982 MAGMA experiment^{5,6} for PmS phases. Out of 51 line segments at plausible PmS ranges from the three sites, only two instances of PmS emerged. The two PmS phases appear on unrotated horizontal components in which the raw horizontal data have not been linearly combined to yield motions along an azimuth different from either of the two original orientations of the geophones at site II (Fig. 2b) from shots on lines 7 (Fig. 3b) and 8. The point of conversion in the MAGMA data lies between an off-axis seamount at $12^{\circ}50'$ N and a segment of ridge that showed active hydrothermal venting one year before the MAGMA experiment⁷.

Figure 3c shows disturbed propagation to site III from shots on the eastern part of line 22. At ranges between 15 and 25 km, the P arrivals on the vertical component in Fig. 3c have a remarkable high-frequency character. Rays at these ranges have turning points at middle-to-lower crustal levels and thus come close to the anomalous Moho, which strongly affects grazing P waves. In general, heterogeneities adjacent to ray paths affect low-frequency waves more than high-frequency waves, and I suggest that this results in the observed high-pass filtering effect. The spectrum of the seismic waves, which is dominated by the fundamental and third harmonic of the bubble pulse, enhances this effect. Therefore, these observations are also consistent with the presence of a strong scatterer at or near the Moho with nearly the same geographical location as the conversion point responsible for the PmS phase at site II.

Of all the instances of PmS presented here, the ROSE observations are the most difficult to explain. Even a very soft serpentine layer with sharp boundaries did not yield PmS at the large ranges seen in the ROSE data. Only the results using a model with a layer of extremely low rigidity at the Moho, interpretable as a sill of partial melt, resembled the data. Figure 4 shows one of these models. The melt layer is 10-m thick and has a P velocity of 4 km s^{-1} and an S velocity of 0.1 km s^{-1} . These values may vary somewhat without much affecting the synthetic seismograms if the rigidity of the melt layer is very low. The PmS phase has the same travel times and distances and is stronger for a 100-m-thick melt layer. The most important result of this modelling is that the PmS phase now extends significantly beyond the 20-km range, in accordance with the ROSE observations. No model without an extremely low rigidity layer produced this feature. Models with smooth, transitional Moho structures, except for interruption by a melt layer, require a thicker (50–100 m) melt layer to yield a strong PmS phase. Velocity gradients below the melt layer can intensify the PmS phase locally.

The amplitudes in the ROSE data are larger than the model predicts. It is most likely that the melt body has only a small lateral extent and that the shear waves are generated on transmission of upgoing mantle P waves. Unfortunately, the propagation effects of this form of heterogeneity are very difficult to model numerically. The absence of melt where the P waves enter the mantle allows stronger P-wave transmission, resulting in stronger long-range PmS than is predicted by the laterally homogeneous model. The Moho structure below the melt layer affects the pattern of amplitudes of PmS by intensifying the incident P waves where caustics form in the gradient structure. This effect may account for the close association of the distant PmS observations with the near PmP caustic in the ROSE data. (PmP designates P-to-P reflection by the Moho.) Figure 4 also

shows that a melt body is highly reflective to P waves at short ranges, and these reflections should be quite prominent as bright spots on the Moho in multichannel seismic (MCS) data.

The ranges at which PmS phases are seen in the MAGMA data set on lines 7 and 8 would be consistent with true PmS. The intervening low-velocity ridge structure, however, almost certainly forces the PmS ray paths to be transmitted up through the Moho instead of being reflected. In the most likely case, that the P-to-S conversion occurs on transmission, only the melt-layer model is acceptable.

It is very unlikely that heterogeneities elsewhere in the crust are responsible for the converted shear waves. A near-sea-floor heterogeneity (perhaps a highly altered fault zone) might be placed to scatter nearly horizontally travelling shear waves with about the right delay after the P arrival. These shear waves would show up, however, on the vertical components prominently, contrary to the observations, and they would probably appear at almost all ranges. A low-rigidity dyke structure at the base of the crust would be oriented incorrectly with respect to

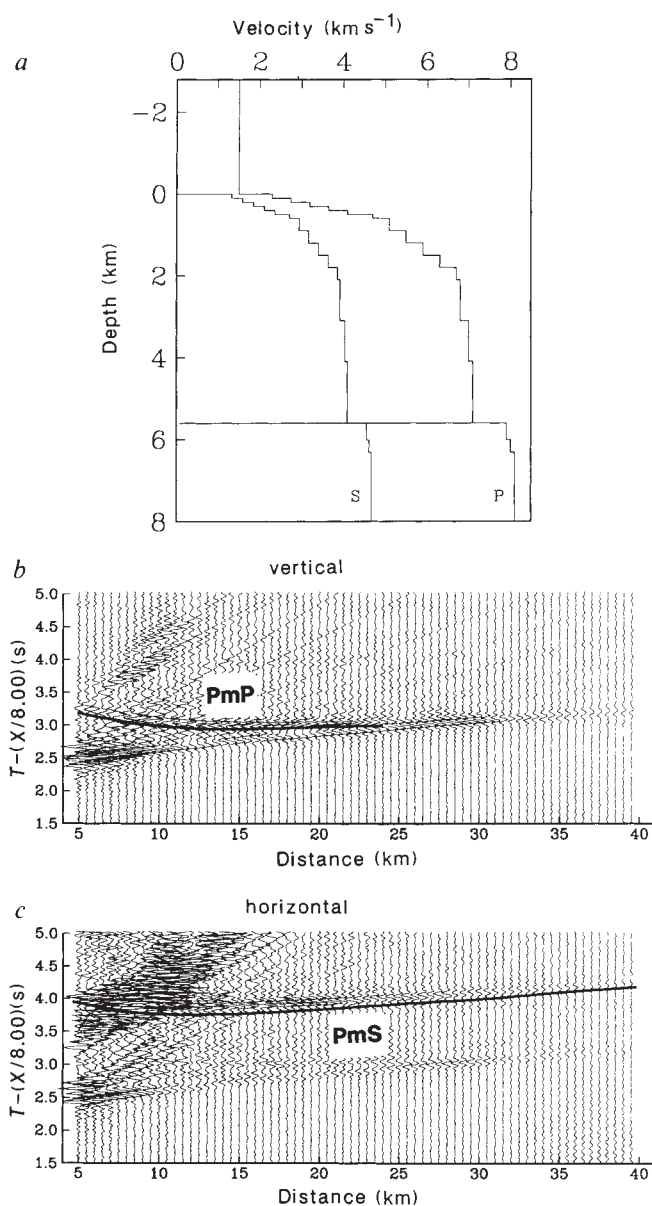


FIG. 4 a, The model and b, c, synthetic seismograms for melt layer at Moho. Water depth 2.8 km. PmS now extends beyond 20 km, mostly diffracted along top of melt layer. Note prominent precritical PmP reflection.

the incident P waves to generate high-amplitude converted shear waves at the observed ranges and times.

It is improbable that Moho topography would be varied enough to intensify PmS sufficiently. The required increase in amplitude is, conservatively, a factor of ten beyond the 20-km range. I assume that this increase requires an expansion of the shear-wave Fresnel-zone area at the Moho (for S-wave propagation from the OBS to the Moho) by roughly the same factor. This requirement results in Moho topographic changes of at least 2-km height over distances of the order of 5 km using the most optimistic assumptions for this mechanism of intensifying PmS. MCS surveys of crustal thickness do not show such large changes over distances this sort^{8,9} except at fracture zones¹⁰. Moho geometries that are less than optimal for this mechanism must cause implausibly abrupt variations in crustal thickness, which would be evident in the seismic data. I conclude that the shear waves are generated by a relatively horizontal body of low rigidity at the Moho, almost certainly a melt-filled sill.

Freezing of the melt bodies at the base of the crust may give rise to a time-varying seismic structure. Repeated OBS or MCS surveys should show these changes if the time scales involved are not too great. For a 10-m-thick sill, the freezing is on the order of a year¹¹. MCS surveys may be more useful in providing extensive surveys for the distribution of melt bodies, but they should be supplemented by OBS data for certain identification of melt. There should be unusual range-dependent variations, however, in the P-to-P reflectivity of the anomalous Moho for sufficiently large source-receiver separations. If these variations are discernible, they may provide a useful diagnostic for the presence of melt.

The observation of sills as far as 22 km from the ridge is consistent with a diapir-and-dyke model of magma transport to the crust over broad zones of upwelling and melting, in agreement with recent geodynamical studies¹². It is reasonable that the most significant compositional boundary in the crystalline oceanic lithosphere would deflect ascending melt. The deepest crustal material close to the ridge is too hot and ductile to permit the melt to ascend by fracturing, so that the rheological and density contrasts at the Moho force the motion of the melt to be close to horizontal. Trapped melt may freeze in place, possibly creating coarse igneous layering in the lower crust. Geological studies of ophiolites¹³, which are thought to be slices of oceanic crust emplaced on land, show prominent igneous layering in sections corresponding to the lower crust¹³. Further from the ridge, the crust may become sufficiently cold and brittle that part of the melt escapes by dyke formation (fracture) to the sea floor, forming an off-axis seamount. The seamount at 12° 50' N is ~5 km from the ridge and could have formed in this way. Melt viscosity and Moho topography are the predominant influences on migration of melt at the Moho, and with sufficient overpressure, melt transport distances and velocities may be large¹⁴. Thermal expansion of the mantle causes the Moho boundary to slope downwards away from the ridge to provide a mechanical funnel for melt incident on the Moho. It is very likely that a nearly horizontally oriented plumbing system guides melt near the end of its ascent to collect in the magma chambers observed¹⁵ at the ridge. □

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Modelling the advection of herring larvae in the North Sea

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THE number of fish at the age of first capture in a fishery (recruitment) is dependent on the production of eggs by the parent stock and the survival of early life stages (eggs, larvae and juveniles). In many pelagic fish species the survival of larvae depends on transport from spawning to nursery areas¹. To investigate larval transport processes for North Sea herring (*Clupea harengus* L.) we have modelled in three dimensions the advection of autumn-spawned larvae during the winter of 1987-1988 and compared the results with sequential field data on the actual distribution of larvae. Circulation in the North Sea is predominantly wind-driven during the winter, and in 1987-1988 anomalous atmospheric conditions caused a reduction in cyclonic circulation and unusual transport of larvae from northern North Sea and west of Scotland spawning areas. Predicting variations in recruitment in advance of fishery legislation has always been difficult and the collapse of North Sea herring populations during the mid-1970s is believed to have been due to a period of several years of low recruitment coupled with high fishing activity². Our results suggest that a better understanding can be achieved with the aid of environmental modelling.

Herring lay eggs on the sea bed off the north and west of Scotland and along the UK coast of the central and northern North Sea, during August and September each year³⁻⁵. Further spawning takes place in the Southern Bight and English Channel between November and January^{3,6}. The larvae hatch within 7-14 days of egg deposition and develop within the plankton until April-June, when they metamorphose into juvenile (0-group) fish⁷. The literature on the winter distribution of herring larvae in the North Sea indicates that larvae from many spawning sites are carried eastward by the water currents towards the continental coast, although some remain in the western North Sea⁸⁻¹¹. The abundance of autumn-spawned larvae in the southwestern North Sea during the International Young Fish Survey (IYFS) (ref. 12) in February has been correlated with recruitment to the North Sea adult populations during the following years. The absence of larvae in some years has been linked with anomalous circulation of the North Sea⁹.

Between August 1987 and March 1988, nine laboratories from Denmark, the Federal Republic of Germany, Norway and the UK participated in the Autumn Circulation Experiment (ACE) (ref. 13), an oceanographic and biological study in the North Sea and off the west of Scotland. One objective of ACE was to examine the distribution of larvae in relation to the water circulation. Field observations of larval distribution between September

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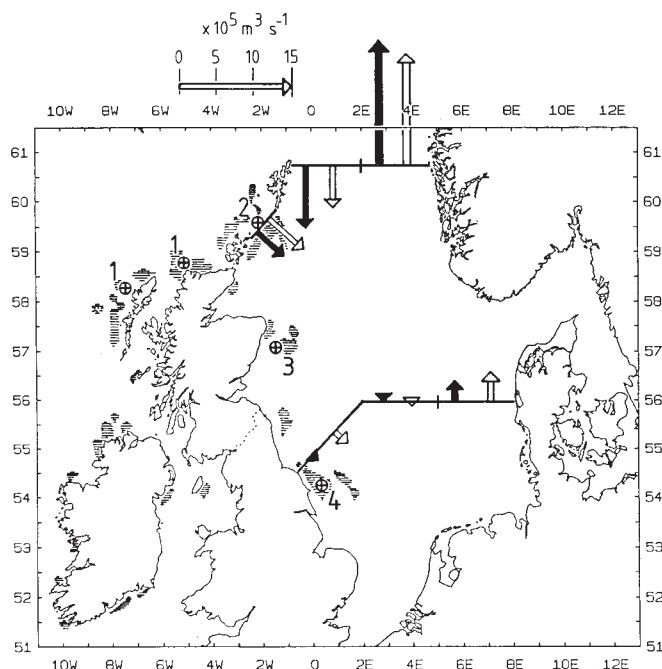


FIG. 1 Geographical distribution of autumn (August–October) spawning areas for herring in the North Sea and west of UK (hatched areas). Numbers refer to starting points for tracers introduced into the three-dimensional advection model: 1) northwest Scotland; 2) Orkney Isles; 3) northeast Scotland; and 4) Yorkshire coast. Open arrows indicate the long-term mean (1969–1982) volume transport during September–December through sections in the North Sea; closed arrows indicate mean volume transport during September–December 1987, calculated from the three-dimensional circulation model.

1987 and March 1988 were compared with distributions predicted by a three-dimensional advection model.

A system comprising two models simulated the advection of herring larvae during ACE (ref. 14). First, a three-dimensional nonlinear numerical model simulated the circulation of the North Sea and adjacent shelf areas¹⁵. The horizontal grid size of the model was 20 km and the time step was 40 min. The circulation model was forced by the M2 tide, climatological mean summer (May–October) and winter (November–April) stratification, and three hourly surface wind stress and air pressure fields. Daily values of current velocity and variance were calculated by integrating over two tidal cycles. Secondly, the daily three-dimensional current velocity and variance field served as the input to an advection model. The latter model incorporated horizontal and vertical advection, as well as diffusion which was parameterized using the daily current variance. Herring larvae were represented in this model by tracer particles which were programmed to undertake diurnal vertical migrations.

The simulations of herring larvae transport began in September 1987 and continued until the end of February 1988. Four herring spawning areas (starting points for the tracers) were considered (Fig. 1). Tracers were introduced into the model over periods of 10 days. This simulated hatching commenced on 3 October off the Yorkshire coast and on 3 September in all other areas, in accordance with the field estimates of mean hatching dates obtained from the International Herring Larvae Surveys (IHLS) (ref. 16). The total number of tracers introduced in each area was weighted according to the estimates of total production obtained from the IHLS in 1987 (ref. 17).

The vertical position of tracers in the water column can influence their horizontal advection¹⁴. An active vertical component was therefore imposed on the tracer trajectories to simulate the vertical migrations of herring larvae. In general, larvae

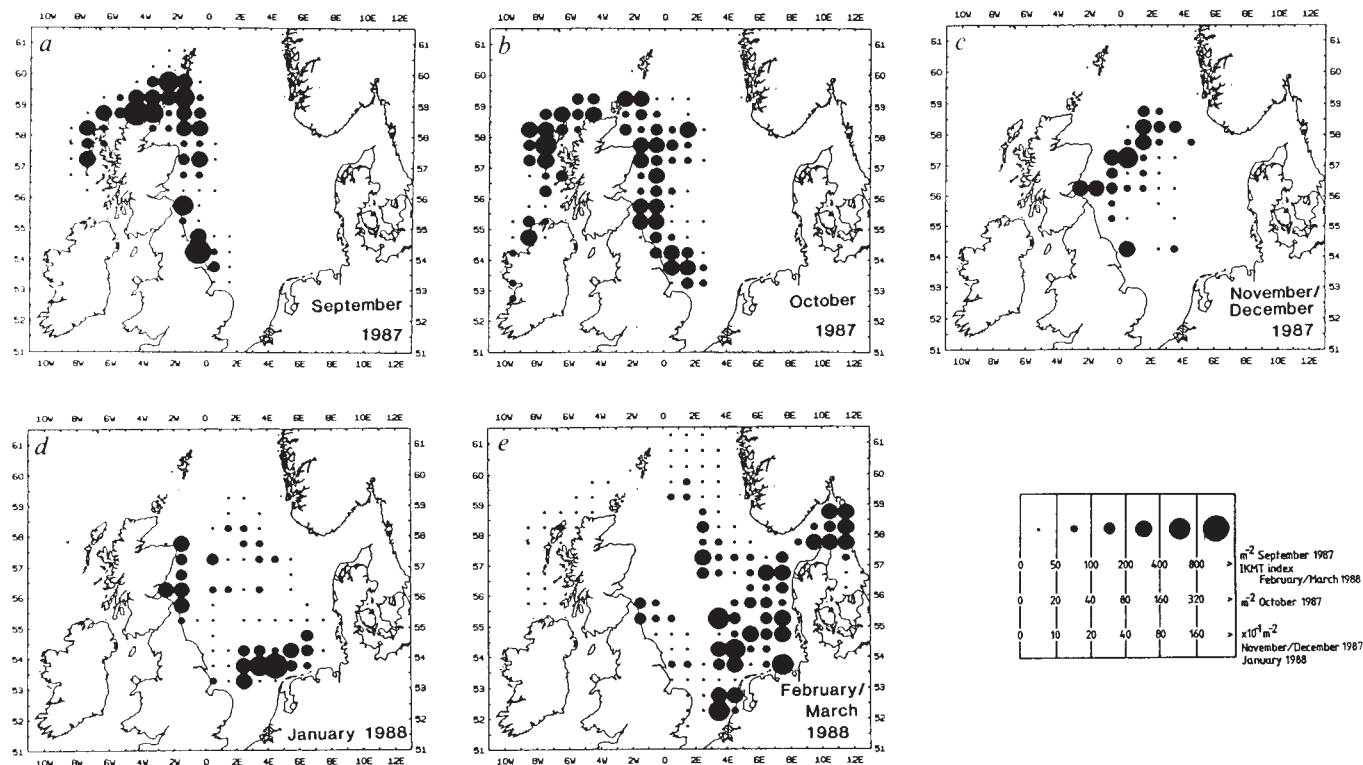
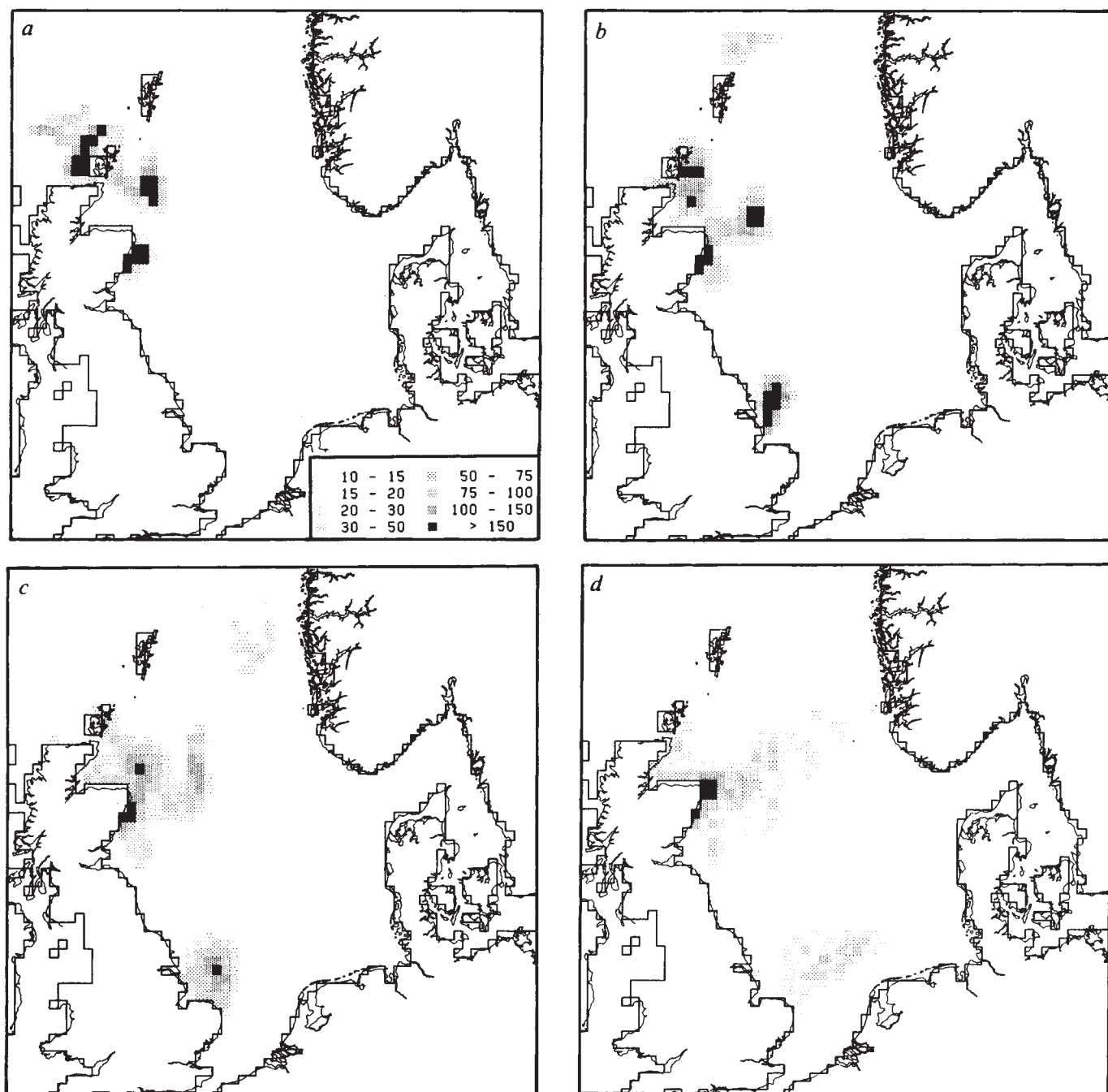


FIG. 2 Field data on the depth-integrated distributions of herring larvae. Data have been averaged over 30' latitude $\times$ 60' longitude rectangles for presentation. *a*, 1–30 September 1987; *b*, 1–30 October 1987; *c*, 20 November–10 December 1987; *d*, 2–27 January 1988; and *e*, 1 February–16 March 1988. Larvae sampled with: *a* and *b*, a Gulf III (0.03 m² mouth, 250 μ m

aperture mesh)²³, some data from IHLS data base²⁴; *c* and *d*, a Methot-Isaacs-Kidd trawl (5 m² mouth, 1.5 mm aperture mesh)^{25,26}; *e*, an Isaacs-Kidd trawl (10 m² mouth, 2.5–5 mm aperture mesh)^{27,28}, North Sea data from IYFS data base²⁹. The northwestern North Sea was not sampled in February 1988 because of incorrect rigging of gear.



undertake diurnal vertical migrations, moving towards the surface during daylight hours and becoming deeper and more dispersed at night (refs 18 and 19; P.M. *et al.*, unpublished results). In the absence of a comprehensive theoretical model of larval migrations, a simplified scheme was used in the advection model: in water deeper than 40 m, the lower level of migration was stipulated to be at 0.6 times the water depth, with the upper level lying 20 m higher. At dusk, the tracers were moved to the lower level over a period of 6 h and remained there until dawn, whereupon they were moved to the upper level, again over 6 h. In water shallower than 40 m, the upper level of migration was fixed at 4 m and the lower level remained the same as for deeper waters. At every time step, each tracer was displaced by the current velocity vector at the actual depth of the tracer.

The atmospheric air pressure and wind stress fields which are the main driving forces of the North Sea circulation during winter showed remarkable deviations from the climatological

means during October, November and December 1987 (J. O. Backhaus *et al.*, unpublished data). Strong southerly and southeasterly winds, lasting for periods of days to weeks, alternated with the more usual westerly airflows. This unusual wind field reduced the cyclonic circulation in the North Sea. Results from the circulation model indicated that between September and December, water mass transport into the northwestern North Sea between the Orkney and Shetland Isles was approximately 75% of the long term (1969–1982) mean inflow (D. Hainbucher, *et al.* unpublished data) (Fig. 1), whereas inflow at the western edge of the Norwegian Trench between Shetland and Norway increased to ~140% of the long term mean over the same period. During this period, the influx of northern North Sea water into the southern North Sea was reduced by approximately 50% (compared with the long-term mean), and hence the flushing of the southern North Sea during autumn 1987 was anomalously low.

The spatial distributions of tracers (two-dimensional depth

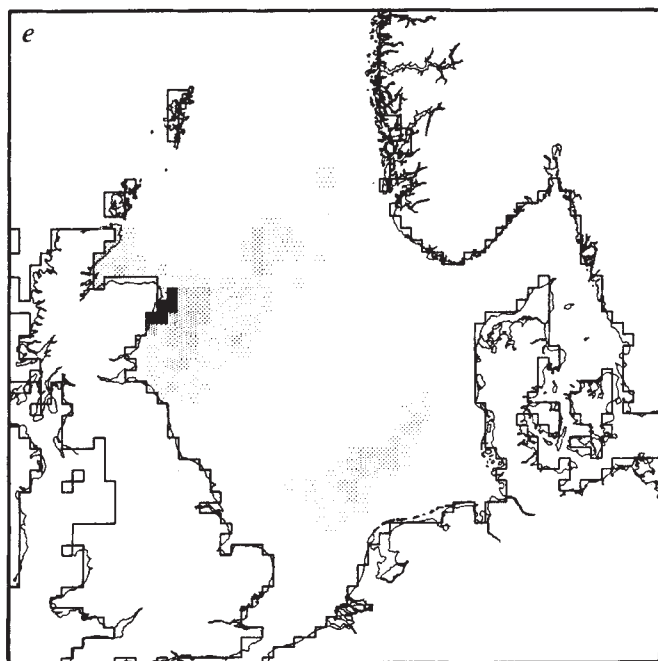


FIG. 3 Two-dimensional (depth-integrated) distribution of tracers from all spawning sites at various dates after introduction into the advection model. a, 30 September 1987; b, 30 October 1987; c, 4 December 1987; d, 28 January 1988; and e, 28 February 1988.

integrated) were taken from the model at 10-day intervals during September and October and at four-day intervals between November and February for comparison with the field study data on herring larvae. No attempt was made to include mortality into the tracer model, so that absolute abundances should not be compared with the field data on the concentration of herring larvae. A comparison, however, of relative distributions of larvae (Fig. 2) and tracers (Fig. 3) is valid. Common features in both the modelled and observed distributions were the persistence of tracers and larvae off the east coast of Scotland throughout the winter, the lack of southerly spreading into the central North Sea, and the eastwards spreading of larvae and tracers from the Yorkshire coast into the German Bight during January and February.

There were two main discrepancies between the modelled and observed distributions of larvae in the central North Sea. First, the rate at which larvae spread through the German Bight and along the Danish coast was more rapid than estimated by the model. Secondly, larvae were present in the inner Skagerrak during surveys in February but no tracers reached the Skagerrak in the simulation. Regular sampling across the mouth of the Skagerrak, along a line running from southern Norway to northern Denmark, indicated that significant numbers of 28–33 mm larvae first arrived in this area in late January²⁰.

Three observations lead us to believe that most of the larvae arriving in the Skagerrak probably originated from the central North Sea spawning areas. First, local spawning in the Skagerrak is insufficient to account for the concentrations of larvae found in February. Secondly, the mean length of the larvae caught in the Skagerrak in February (30–35 mm) was greater than that of larvae in the northern North Sea (23–26 mm), but similar to that of the larvae found in the southern North Sea in January (28–33 mm). Finally, larvae in the northern North Sea during January and February were associated with water of high salinity ($>35 \text{ g kg}^{-1}$) and nitrate concentration of approximately $7\text{--}8 \mu\text{M}$, but water with these characteristics never extended eastwards further than 4°E , and certainly never entered the Skager-

rak. The water in the Skagerrak was characteristically of low salinity ($<33 \text{ g kg}^{-1}$) and had a concentration of nitrate $>12 \mu\text{M}$.

Bartsch¹⁴ has shown that the choice of vertical migration model can have a considerable effect on the simulated advection of larvae. An additional simulation of the advection of larvae from the Yorkshire coast spawning site was therefore carried out with a vertical migration pattern in which the larvae were closer to the surface (5 m below the surface during daylight and 15 m at night). The distribution in January with the modified vertical migration pattern was similar to that in the original simulation, but during early February the tracers were rapidly carried northwards along the Danish coast and into the Skagerrak (Fig. 4), roughly corresponding with the field observations of the distribution of larvae. These results suggest that our original scheme for simulating vertical migrations, which was based on observations made in northern waters, was inappropriate in the southern North Sea. Investigations carried out during ACE with a 5-m² net and other opening and closing gears did indicate that vertical migrations of larvae followed different patterns in the shallow ($<45 \text{ m}$) waters of the southern North Sea from those in the deeper waters of the north.

The eventual fate of larvae in the northern North Sea after February 1988 is not known. The advection model and data on the trajectories of satellite-tracked drifting buoys released during ACE suggest that some may have been carried out of the North Sea in the north-flowing Norwegian coastal current and lost to the North Sea population. Simulations under long-term mean circulation conditions indicate that the northern North Sea larvae would be expected to reach the Skagerrak by February and remain within the North Sea ecosystem¹⁴. This did not happen in 1987–1988 and we therefore anticipate abnormal recruitment of the 1987 year class to the northern spawning populations in 1990. This expectation is consistent with the conclusion of Bartsch¹⁴ that 'favourable circulation' in the North Sea is a necessary, but not sufficient, prerequisite for good recruitment.

The data from ACE support the use of three-dimensional circulation and advection models for studying the advection and dispersion of vertical migrating fish larvae. Analysis of modelled advection routes over several year classes confirms

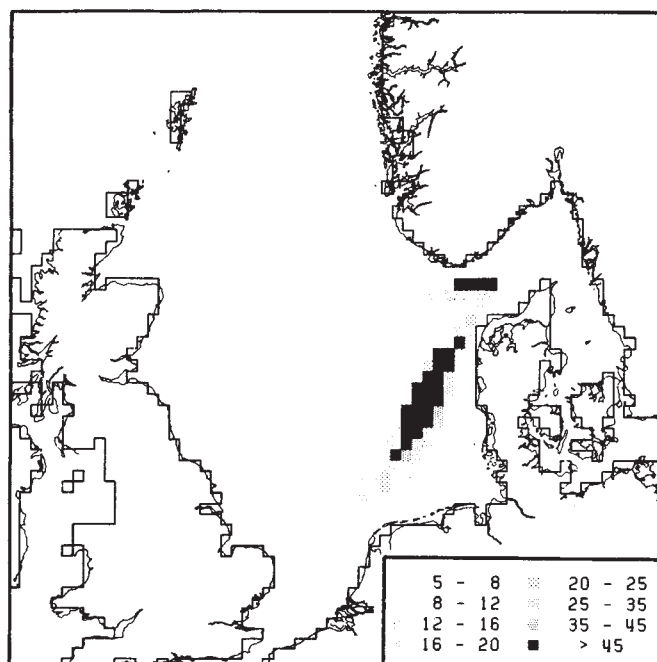


FIG. 4 Two-dimensional (depth-integrated) distribution on 28 February 1988 of tracers from the Yorkshire coast spawning area only, modelled with tracers having a reduced amplitude of vertical migration (5–15 m).

that eastwards dispersion of larvae across the North Sea is the predominant transport phenomenon, but that some larvae invariably remain along the UK east coast¹⁴. In all years, there is mixing between tracers from widely separated spawning areas. Because the winter circulation in the North Sea is mainly wind-driven^{21,22}, the pattern of larval dispersion will vary from year to year, depending on the meteorological conditions. □

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Presynaptic spike broadening reduces junctional potential amplitude

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PRESYNAPTIC modulation of action potential duration may regulate synaptic transmission in both vertebrates¹ and invertebrates^{2–4}. Such synaptic plasticity is brought about by modifications to membrane currents at presynaptic release sites, which, in turn, lead to changes in the concentration of cytosolic calcium available for mediating transmitter release. The 'primitive' neuromuscular junction of the jellyfish *Polyorchis penicillatus* is a useful model of presynaptic modulation. In this study, we show that the durations of action potentials in the motor neurons of this jellyfish are negatively correlated with the amplitude of excitatory junctional potentials. We present data from *in vitro* voltage-clamp experiments showing that short duration voltage spikes, which elicit large excitatory junctional potentials *in vivo*, produce larger and briefer calcium currents than do long duration action potentials, which elicit small excitatory junctional potentials.

Synaptic modulation by changes in motor neuron action potential duration serves to synchronize the swimming contractions of the hydrozoan jellyfish *Polyorchis penicillatus*. Action

potentials can arise in any depolarized part of the circular, electrically-coupled network of swimming motor neurons^{5,6}. As action potentials propagate into progressively more polarized regions of the network, their duration progressively decreases, the excitatory junctional potentials (e.j.ps) elicited in the swimming muscle become larger and the delay to generation of a muscle action potential is reduced⁶ (Fig. 1). This decreased delay automatically compensates for the conduction time of the motor spike through the network. These findings are paradoxical, since contemporary models⁷ of calcium-mediated release of transmitters predict that postsynaptic potential amplitude should be positively correlated with action potential duration.

As *in vivo* study of membrane ionic currents in the motor neuron network of *Polyorchis* is problematic, because extensive electrical coupling makes voltage-clamp recording ineffective, to further investigate the above phenomena we have developed a method to isolate these cells in primary culture⁸. We have used the whole-cell recording technique to characterize the ionic currents regulating action potential duration and the Ca^{2+} currents which probably mediate transmitter release in the system.

Current-clamp recordings from isolated motor neurons showed that the duration of stimulated action potentials increased with membrane baseline depolarization (unpublished data). We presume this resulted from the steady-state, depolarization-dependent inactivation of an 'A-like', rapidly activating, transient potassium current ($I_{k\text{-fast}}$) which is responsible for action potential repolarization. Voltage-clamp data showed that $I_{k\text{-fast}}$ peaked within 2 to 15 ms, decayed with a time constant of about 200 ms (Fig. 2a) and was half-inactivated at a prepulse potential of -47 mV ($N=4$; Fig. 2b). The normal *in vivo* range of resting membrane potentials (-25 mV to

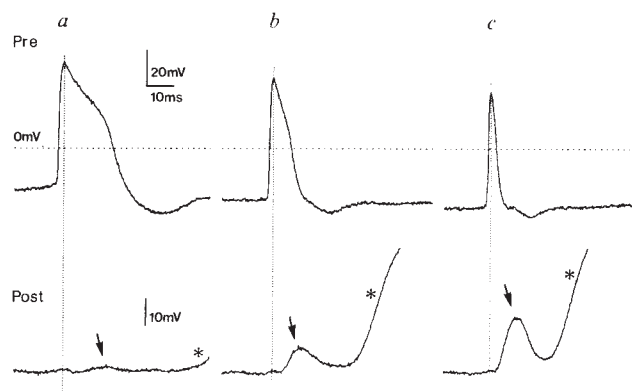


FIG. 1 Motor neuron action potential duration is negatively correlated with muscle e.j.p. amplitude, *in vivo*. These simultaneous pre- and postsynaptic recordings were made across the swimming motor neuron-myoeptithelial cell junction in a semi-dissected preparation of the bell margin from *Polyorchis penicillatus* (methods described in ref. 6). Electrodes were positioned ~ 50 μm apart. Motor neuron action potentials were spontaneously initiated from random sites around the nerve-ring. *a*, When the initiation site was close to the recording electrodes, a broad action potential (upper trace) was seen to arise from a depolarized baseline and elicited a very small e.j.p. (bottom trace, arrow) with a long delay to the muscle action potential (bottom trace, asterisk). *b*, When the initiation site was more distant from the electrodes, the recorded action potential propagated into a more polarized region of the electrically-coupled network, had a shorter duration and elicited a larger e.j.p. (arrow) with a shorter delay to the muscle action potential (asterisk). *c*, An action potential which had propagated from the most distant initiation site to the recording electrodes was seen to arise from the most polarized baseline, had a very short duration and produced the largest e.j.p. (arrow) with the shortest delay to the muscle action potential (asterisk). It is important to note that muscle action potentials occurred at a considerable delay after the e.j.p. since the distance between the recording and muscle spike-initiation sites was large (ref. 6). Electrodes were filled with 2 M potassium acetate. Bath solution was natural sea water at 20°C . Data were stored on FM tape, then digitized at 200 μs intervals for plotting.

-50 mV) was within the steeper portion of the steady-state inactivation curve for this current, suggesting that I_{k-fast} was modulated at resting potentials where spike duration varied. A slower outward current, I_{k-slow} , was revealed at more depolarized holding potentials (≥ -30 mV; Fig. 2a), where I_{k-fast} was almost completely inactivated. This slow current activated within 200 ms and decayed e -fold in 1–2 s. Tetraethylammonium at 100 mM in the electrode completely blocked both currents. Externally applied 4-aminopyridine at 5 to 10 mM reduced I_{k-fast} by about 40% after 30 min ($N=8$), while I_{k-slow} was largely unaffected by this treatment ($N=3$). Both these compounds

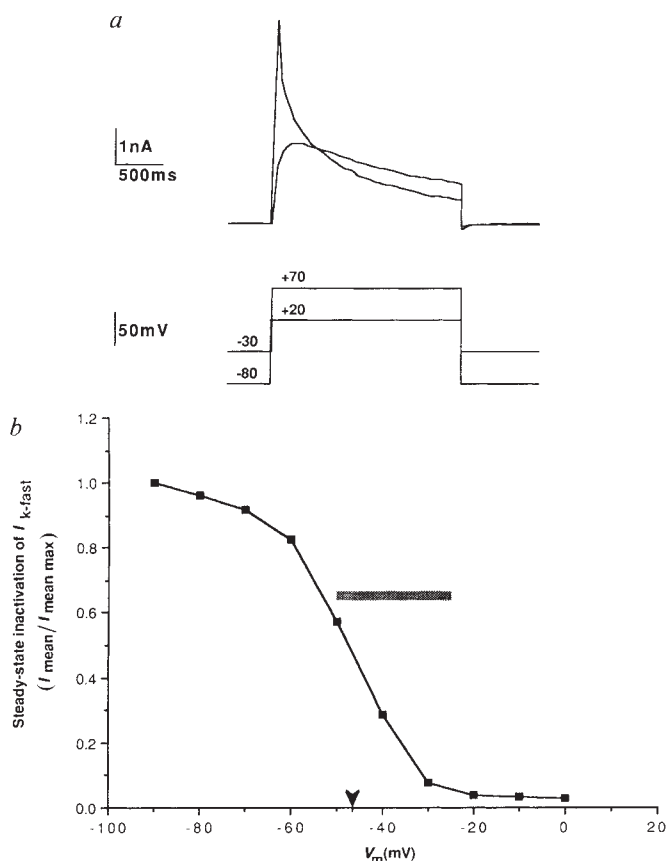


FIG. 2 Potassium currents in isolated motor neurons. a, I_{k-fast} and I_{k-slow} responses to 100 mV depolarizations applied for 2 s from holding potentials (V_h) of -80 mV and -30 mV, respectively. Current traces were digitized from chart recordings using a 45 ms sampling interval. Voltage protocols were redrawn. b, Plot of the voltage-dependence of steady-state inactivation of I_{k-fast} . A 'pre-pulse/pulse' protocol was applied from $V_h = -80$ mV. A 2-s pre-pulse was applied at command voltages incremented in 10 mV steps; a test pulse to +10 mV then followed. Early peak outward currents elicited by the test pulses were measured, leak-subtracted, and normalized to the largest current obtained; these ratios were plotted against pre-pulse potential. This current was active and modulated at *in vivo* resting potentials (shown by stippled bar above plot). Average maximal peak current, elicited after a prepulse potential of -90 mV, was 2.99 nA. Voltage of half-inactivation (-47 mV) is shown by the arrowhead. $N=4$ except for the last three points on the right which are $N=3$, 3 and 2, left to right. Contamination by I_{k-slow} was assumed to be minimal during the early (<20 ms) phase of the response where measurements were taken, since I_{k-slow} was only weakly activated at the test potential and arose ten times more slowly than did I_{k-fast} . Sodium-free bath solution was used which contained, in mM: choline-Cl, 437; $MgSO_4$, 5.7; $MgCl_2$, 23.3; $CaCl_2$, 9.5; KCl, 7.8; HEPES, 10; KOH, 5.6. Calcium current was blocked with 0.1–0.5 mM $CdCl_2$ in the bath. Whole-cell recording internal solution contained, in mM: Dextrose, 740; KCl, 105; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; KOH, 35. Experiments were carried out at 20–25 °C. Dissociation and culture techniques are explained⁸. The culture medium in the present experiments had been modified and contained, in mM: NaCl, 378; $MgCl_2$, 29; $CaCl_2$, 9.5; Na_2SO_4 , 5.7; KCl, 13.4; choline-Cl, 42; HEPES, 10; NaOH, 5; 50 mg L⁻¹ gentamycin sulphate was added. The pH for all solutions was adjusted to 7.5.

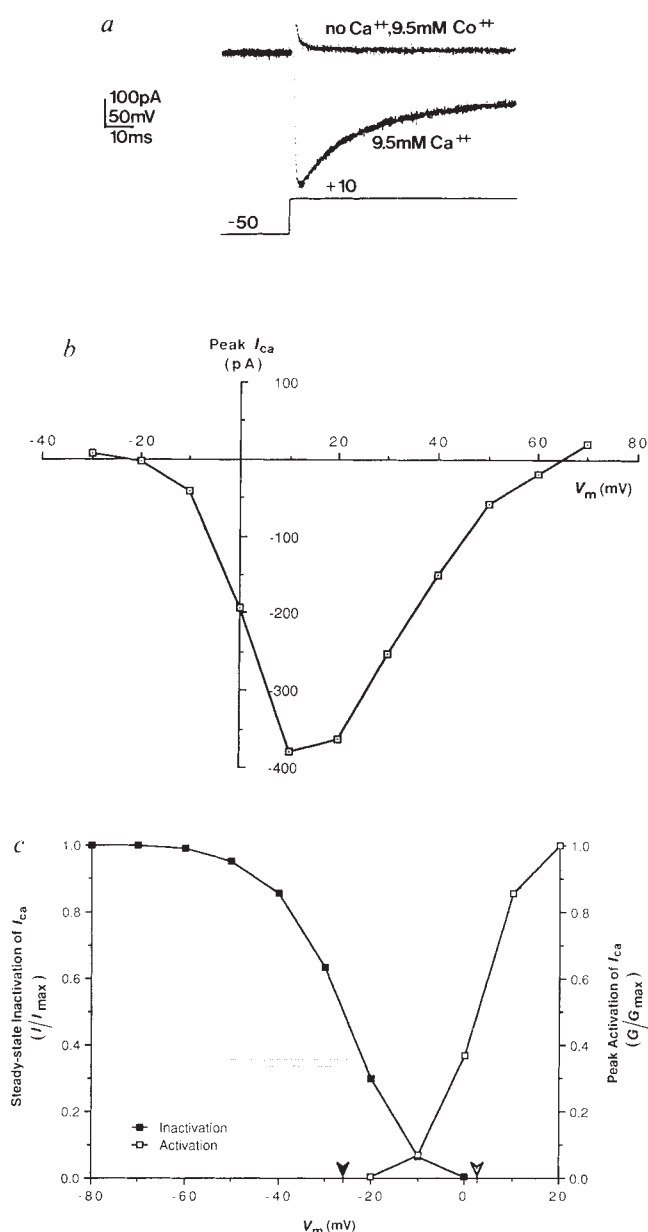
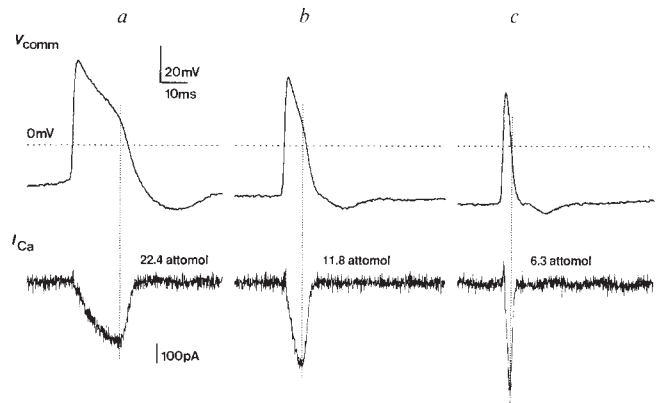


FIG. 3 Calcium current from isolated motor neurons. a, I_{Ca} elicited by a 200 ms voltage step from $V_h = -50$ mV to +10 mV. This current was blocked after 5 min in a bathing medium in which Ca^{2+} was replaced by Co^{2+} . b, Current/voltage (I - V) plot for peak I_{Ca} . Depolarizing test-pulses lasting 80 ms were applied from $V_h = -40$ mV to the voltages shown on the abscissa. Leak-corrected responses from 10 cells were averaged for each voltage point, and the peak inward current was plotted. The reversal at +66 mV suggests contamination of I_{Ca} by some residual outward current, evident at more positive V_m . c, Steady-state inactivation (solid squares, $N=5$) and peak activation (open squares, $N=10$) of I_{Ca} as a function of V_m . Voltages of half-activation (clear arrowhead, +3 mV) and half-inactivation (solid arrowhead, -26 mV) are indicated. Average maximal peak I_{Ca} for inactivation data was -434 pA; average maximal peak Ca^{2+} conductance for activation data was 7.9 nS. The inactivation data were obtained using a pre-pulse/pulse protocol where V_h was -40 mV, the pre-pulse lasted 1 or 2 s and the test pulse went to +10 mV; peak responses were averaged and plotted as a function of pre-pulse potential. The activation curve was drawn from data for b; the driving force and conductances were calculated assuming a reversal potential of +66 mV. The range of normal resting potentials is indicated as a stippled bar. Bath solution was the same as in Fig. 2. Internal solution used in a and for inactivation data in c contained, in mM: CsCl, 140; TEA-Cl, 100; Dextrose, 477; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; NaOH, 28. The I - V data in b, and activation data in c were collected using the following, sodium-free electrode solution, in mM: CsCl, 109; TEA-Cl, 100; Dextrose, 536; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; CsOH, 31.

FIG. 4 Calcium currents elicited from *in vitro* motor neurons by spike-shaped command pulses. *a*, A long duration spike command elicited a slowly developing, small amplitude I_{Ca} . Progressively shorter spike commands (*b* and *c*) elicited a progressively more transient and larger amplitude I_{Ca} . Integrals of these currents are indicated, in attomol (10^{-18} mol) of Ca^{2+} above the current traces. Voltage commands were digitized in 200 μ s samples from the FM-recorded action potentials shown in Fig. 1, filtered at 600 Hz to reduce capacitive noise, and applied with 70% series-resistance compensation (final $R_{ser} = 1-3$ M Ω), through the command input of a List Medical EPC-7 voltage-clamp amplifier. Current responses were digitally recorded on videotape at 44 kHz, then transferred to PCLAMP files at 100 μ s per sample for data processing. Capacitance artefacts and leakage were subtracted using responses to inverted, scaled-down (20%) versions of the spikes, applied at the same holding potential as their full-scale counterparts; similar results were obtained if subtraction pulses were applied at more polarized values for V_h . Contaminating, Ca^{2+} -independent currents were subtracted using leakage- and capacitance-corrected responses obtained from the same cell in Ca^{2+} -free, Co^{2+} -substituted saline. All solutions as for Fig. 3b. Experiments done at 20 $^{\circ}C$.



applied externally caused spike prolongation in current-clamp recordings.

An inward calcium current (I_{Ca}) was recorded from voltage-clamped, cultured motor neurons, which could be eliminated by the replacement of external Ca^{2+} with Co^{2+} (Fig. 3a) or by the addition of 0.1–0.5 mM $CdCl_2$. The I_{Ca} activated rapidly, reaching a peak of several hundred picoamperes within 4 ms, and inactivated with a time constant of 15 to 25 ms. In other systems, calcium currents at presynaptic sites⁷ normally show slower activation and inactivation. A second time constant of decay (60–70 ms) could be measured suggesting the presence of an additional, smaller and more slowly inactivating component of I_{Ca} . Figures 3b and c show the voltage-dependence of peak I_{Ca} , peak G_{Ca} activation and peak I_{Ca} steady-state inactivation. It is important to note that these motor neurons have a compact morphology, lacking long or narrow processes⁸, and that the synapses they make onto myoepithelial cells are located throughout the neurons⁹. Thus, there is good electrical access to the synaptic sites. We have assumed that the large, transient Ca^{2+} current we observed mediates transmitter release in these motor neurons, although it is possible that a smaller I_{Ca} component may be involved.

To determine the dynamics of voltage-gated calcium influx when action potentials of varying duration invade a presynaptic release site, we stimulated motor neurons *in vitro* using voltage commands shaped like action potentials. Figure 4a shows that a long duration action potential elicited a small amplitude, slow calcium current which peaked during the slow repolarizing phase. The same potential *in vivo* had evoked a small amplitude e.j.p. (Fig. 1c). In contrast, a short duration action potential produced a larger, more transient calcium current (Fig. 4c) and, *in vivo*, had elicited a larger e.j.p. (Fig. 1c). Voltage-clamp stimuli of intermediate durations produced calcium currents of intermediate amplitude and time to peak (Fig. 4b). Correspondingly, e.j.p. amplitude has been recorded *in vivo* as varying continuously with action potential duration (ref. 6; Fig. 1).

The amplitudes and time courses of calcium currents seen using simulated action potentials can be explained in the following way. The holding potential preceding the spike determines the number of calcium channels available for activation and, in part, the peak calcium current elicited by the pulse. Thus, brief spikes arising from a more polarized baseline will be able to activate more calcium channels than prolonged spikes arising from a more depolarized baseline. The rising phase of the spike then activates all available calcium channels (Fig. 4 top; Fig. 3b, c) but no current flows until a net inward driving force develops, during repolarization. The rapid repolarization of short duration action potentials causes the driving force on Ca^{2+} to increase at a far greater rate than channels are closing and inactivating, resulting in a large, brief calcium current analogous

to a 'tail' current. In contrast, the slower rate of repolarization of long duration action potentials is associated with a slower development of the electrical driving force, thus resulting in a more slowly developing calcium current of smaller amplitude.

Measurements of the integrals of I_{Ca} responses reveal that despite the larger peak I_{Ca} elicited by short duration action potentials, long duration action potentials produced a larger total Ca^{2+} influx (Fig. 4). How does a small but rapid entry of calcium release more transmitter, as judged by the amplitude of the e.j.p., than a larger but slower entry of calcium?

There is overwhelming evidence that fast transmitter release depends on the calcium concentration at cytosolic binding sites which regulate the rate of vesicle fusion to the plasma membrane^{7,10}. Calcium concentration at these sites, which are thought to be located just beneath the membrane^{7,10}, is in turn determined by the difference between the rate of calcium influx and the rate of Ca^{2+} removal by diffusion, active pumping, and sequestration⁷. It is possible that the Ca^{2+} sinks in *Polyorchis* motor neurons are very effective and that they counter the increase in submembranous $[Ca^{2+}]$ produced by slow Ca^{2+} influx (during long spikes), while allowing rapid influx to cause a large change in submembranous $[Ca^{2+}]$.

Since these experiments were carried out with 11 mM intracellular EGTA, however, it is possible that Ca^{2+} -dependent inactivation of I_{Ca} , if present, was reduced. Such inactivation would cause the long duration spike to elicit a smaller total calcium influx than observed, which might be less than the influx produced by a short duration spike in native conditions. In this case rapid removal of calcium from cytosolic binding sites would not be necessary to explain the effectiveness of short duration spikes in releasing transmitter material.

This study shows that there can be considerable modulation of the time course of the presynaptic calcium transient by variations in the shape of the presynaptic action potential and that this can lead to differential efficacy of synaptic transmission.

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α -Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating

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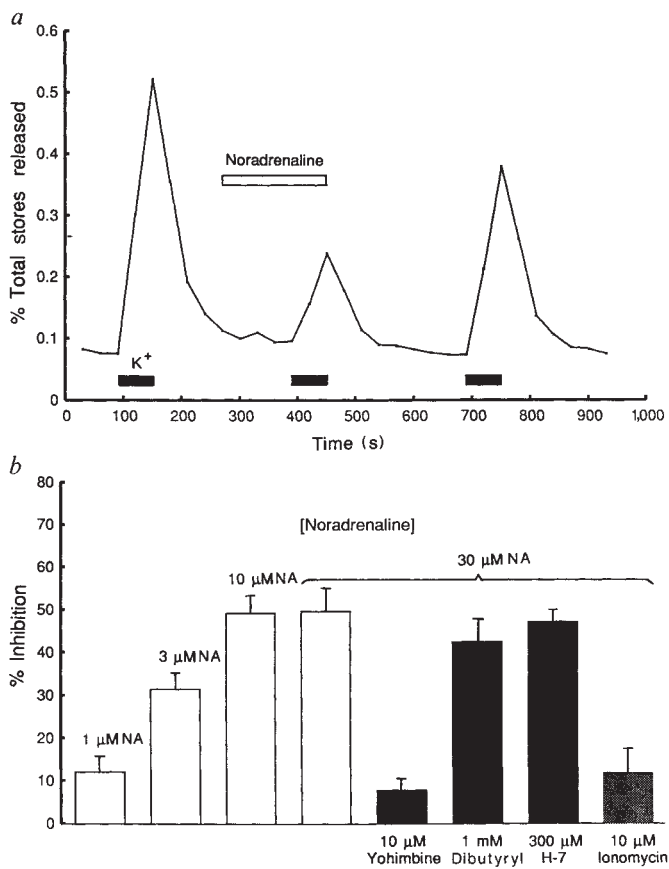
IN sympathetic neurons, catecholamines interact with prejunctional α -adrenergic receptors to reduce delivery of transmitter to postjunctional target organs¹⁻⁴. This autoinhibitory feedback is a general phenomenon seen in diverse neurons containing a variety of transmitters²⁻⁴. The underlying mechanisms of α -adrenergic inhibition are not clear, although decreases in cyclic AMP and cAMP-mediated phosphorylation have been implicated¹⁻⁴ (compare ref. 5). We have studied depolarization-induced catecholamine release and calcium-channel currents in frog sympathetic neurons. Here we show that α -adrenergic inhibition of transmitter release can be explained by inhibition of Ca^{2+} -channel currents and not by modulation of intracellular proteins. Noradrenaline strongly reduces the activity of N-type Ca^{2+} channels, the dominant calcium entry pathway triggering sympathetic transmitter release⁶, whereas L-type Ca^{2+} channels are not significantly inhibited. The down-

modulation of N-type channels involves changes in rapid gating kinetics but not in unitary flux. This is the first detailed description of inhibition of a high-voltage activated neuronal Ca^{2+} channel at the single-channel level. The coupling between α -adrenergic receptors and N-type channels involves a G protein, but not a readily diffusible cytoplasmic messenger or protein kinase C, and may be well suited for rapid and spatially localized feedback-control of transmitter release.

Figure 1 shows the inhibitory effects of noradrenaline (NA) on transmitter release in isolated frog sympathetic ganglia⁷, to facilitate comparisons with measurements of ionic currents in cell bodies (Fig. 2). Noradrenaline strongly and reversibly reduced transmitter outflow evoked by exposure to K^+ (Fig. 1a). The inhibition reached a maximum of ~50% at 10–100 μM NA (Fig. 1b). Inhibition was unaffected by the β -adrenergic blocker, propranolol, but was prevented by phenolamine (10 μM), a general α -adrenergic antagonist, or by yohimbine (10 μM), an α_2 -adrenergic antagonist (Fig. 1b). Application of the α_2 -adrenergic agonist clonidine (at concentrations ≤ 100 μM) had no effect. These pharmacological properties are characteristic of a distinct subtype of α_2 -adrenergic receptor described in other neurons^{8,9}. It has been suggested¹⁻⁴ that NA autoinhibition might involve altered phosphorylation of Ca^{2+} channels or of intracellular proteins, such as synapsin I (ref. 10), by cyclic AMP- or Ca^{2+} -dependent protein kinases. We found, however, that the inhibition by 30 μM NA remained unchanged in 1 mM dibutyl cAMP, or in H-7 (a protein kinase blocker) at a concentration (300 μM) that inhibits several protein kinases¹¹ (Fig. 1b). Transmitter release induced by adding Ca^{2+} in the presence of the Ca^{2+} ionophore ionomycin, bypassing calcium entry through voltage-gated Ca^{2+} channels, was not affected by NA (Fig. 1b; ref. 12). This indicates that noradrenergic inhibition does not depend on mechanisms subsequent to a rise in cytosolic Ca^{2+} but is likely to involve modulation of Ca^{2+} entry.

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FIG. 1 a, Effect of 30 μM NA (open bar) on radiolabelled transmitter release evoked by exposure to 50 mM K^+ (solid bars). b, Characteristics of NA effect. Bars represent means $\pm$ s.e.m. of percentage of NA-induced inhibition in 3–8 experiments. Open bars, dose-dependence of NA-induced inhibition; solid bars, effect of 30 μM NA in the presence of the indicated agents. Dibutyl cAMP or H-7 (1-(5-isquinolinesulphonyl)-2-methylpiperazine) did not significantly affect NA inhibition ($P > 0.05$). Grey bar, NA did not significantly affect transmitter release evoked by Ca^{2+} in ionomycin ($P > 0.05$). **METHODS.** Sympathetic chains (3–4 ganglia) from frogs (*Rana pipiens pipiens*) were incubated for 2 h at 22 °C in Ringer's solution containing (mM): NaCl (128), KCl (2), glucose (10), HEPES (10) (pH 7.3 with NaOH); also added were ascorbic acid (antioxidant), (1.0 mM) pargyline (monoamine oxidase inhibitor) (0.1 mM), and [^3H]-NA (10 $\mu\text{Ci ml}^{-1} \approx 0.2$ μM) (New England Nuclear). After [^3H]-NA loading, the ganglia were enclosed in a chamber and perfused at 1.6 ml min⁻¹ with Ringer's solution containing 10 μM desipramine (reuptake blocker), 10 μM propranolol and 2 mM CaCl_2 . Once a stable baseline of ^3H -release was achieved (30–40 min), 30-s fractions were collected continuously. Sympathetic transmitter release (probably a mixture of noradrenaline and adrenaline³⁶, from neuronal somata^{7,27}) was induced by perfusing ganglia with Ringer's solution containing 50 mM K^+ to directly depolarize the cells, bypassing possible changes in action-potential propagation or duration. Release showed dependence on K^+ (30–80 mM) and extracellular calcium (effector concentration for half-maximum response ≈ 0.5 mM), and complete inhibition by cadmium (50% inhibitory concentration ≈ 10 μM) as expected for exocytosis triggered by voltage-gated Ca^{2+} influx. The amount of release was calculated as the area (A) under each peak after baseline subtraction. The release in the presence of NA (A_{NA}) was expressed as a percentage of the average of release evoked before (A_{con}) and after washing out NA (A_{wash}). Thus, percentage inhibition = $[1 - (2A_{\text{NA}}/A_{\text{con}} + A_{\text{wash}})] \times 100$. For ionomycin-induced release, 10 μM ionomycin was added to the perfusion solution (external free calcium concentration buffered at 0.1 μM with 2 mM EGTA) 10 min before collecting fractions. Release was evoked by exposure to 1 mM free calcium. Peak ionomycin-induced release (0.2–0.4%) was similar to that evoked by 50 mM K^+ .



Noradrenaline inhibits Ca^{2+} entry, as measured by whole-cell Ca^{2+} currents from isolated frog sympathetic neurons (Fig. 2), as it does in other neurons¹³⁻¹⁹. In parallel with the release studies, the inhibition of Ca^{2+} current by NA was antagonized by yohimbine (Fig. 2b) and phentolamine, but not by propranolol. Likewise, clonidine (at concentrations up to 100 μM) had no effect. The inhibition was maximal with 10 μM NA (Fig. 2c; refs 9, 15). Replacement of GTP in the recording-pipette solution with a non-hydrolysable analogue, GTP- γ -S (0.05 mM), largely prevented recovery from the NA-induced inhibition (Fig. 2d); internal GTP- γ -S (0.5 mM) alone mimicked the inhibitory effect of NA (results not shown). Both results suggest involvement of G proteins, as in other neuronal systems^{16,20-22}.

Sympathetic neurons have two types of high-voltage activated (HVA) Ca^{2+} channels, N-type and L-type, which differ in their voltage and time dependence of inactivation and in their single-channel conductance^{6,23-25}. The NA-sensitive current showed partial, but not complete, decay over hundreds of milliseconds^{6,25-27} and a strong dependence on holding potential (Fig. 2e-g), all consistent with inhibition of N-type Ca^{2+} channels.

Unitary current recordings provide the most direct approach to identifying the NA-sensitive Ca^{2+} channel and characterizing the modulatory effect. Although many neurotransmitters inhibit HVA Ca^{2+} currents^{8,9,14-22,24,26}, little is known about the mechanism of inhibition at the single-channel level (see ref. 28). Figure 3a-c shows N-type and L-type Ca^{2+} channels studied in isolation in the same patch-recording using appropriate voltage protocols^{6,23,24}. The N-type channel current illustrated in Fig. 3a is sustained during the depolarizing pulse, but in other recordings it often shows a more pronounced time-dependent inactivation^{6,24-27}. In all cases, however, the N-type channel current inactivates following prolonged (10–20 s) depolarizations of the holding potential^{24,27}. To assess the effect of NA, a series of cell-attached patch recordings were obtained with or without NA (10–100 μM) in the recording pipette. The average N-type Ca^{2+} channel currents were reduced from 0.24 ± 0.09 pA ($n = 22$) to 0.09 ± 0.05 pA ($n = 10$) in 30 μM NA, and to 0.03 ± 0.02 pA ($n = 5$) in 100 μM NA (Fig. 3f-h). The L-type Ca^{2+} current was not significantly changed ($P > 0.05$), although there was some hint of inhibition at 100 μM NA. Noradrenaline did not significantly change unitary current amplitude at any concentration tested (legend to Fig. 4). By contrast, there was a marked change in rapid gating kinetics. The mean open time decreased from 0.87 ± 0.14 ms (control; $n = 23$) to 0.38 ± 0.07 ms in 30 μM NA ($n = 7$), or 0.40 ± 0.09 ms in 100 μM NA ($n = 5$). This more than two-fold abbreviation of N-type channel openings contributes substantially to the large decrease in average current seen overall (Fig. 3f-h). Changes in gating kinetics on a time scale slower than the pulse duration are suggested by an increase in the percentage of blank sweeps from 8% in the control to 24% in 30–100 μM NA.

The coupling between the α -adrenoceptor and N-type channel inhibition does not involve a readily diffusible second messenger, as application of 100 μM NA to the bath had no effect on N-type channels under the patch pipette (Fig. 3i,j; see also ref. 17). In particular, the messenger is unlikely to be cAMP, because dibutyryl cAMP had no effect on N-type channels, whereas it did increase the activity of L-type channels. Protein kinase C is also unlikely to be involved, because phorbol esters increase N-type and L-type Ca^{2+} -channel activity in these cells²⁹.

Our results demonstrate that NA acts through α -adrenoceptors to selectively inhibit the activity of N-type Ca^{2+} channels, and link this inhibition to attenuation of sympathetic transmitter release. Noradrenaline reduces the probability of channel opening by accelerating the rate of channel closing, and possibly slowing the kinetics of opening, without changing unitary channel flux. Because the mechanism of inhibitory modulation of unitary HVA neuronal Ca^{2+} -channel currents has not previously

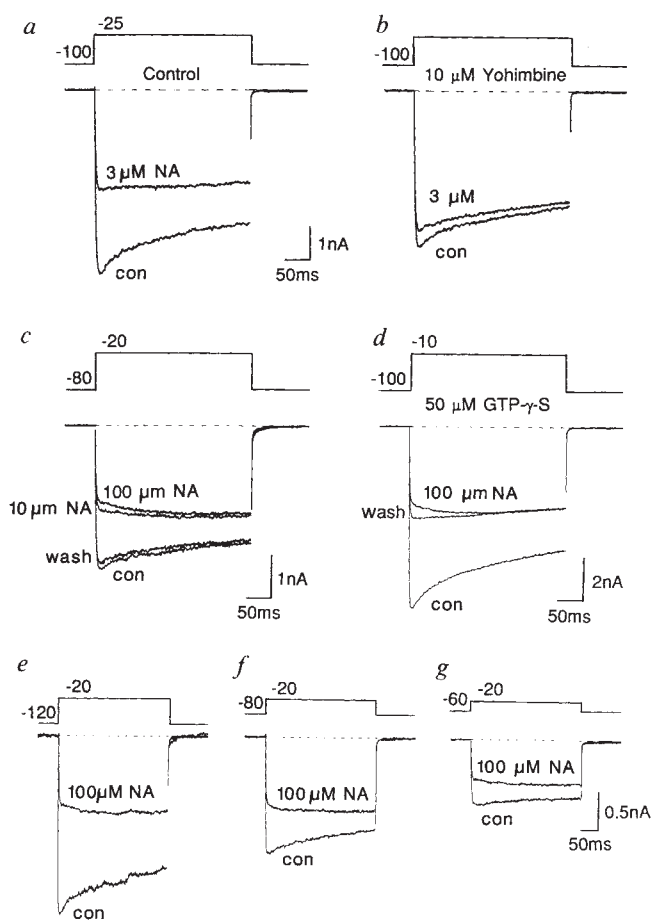


FIG. 2 Noradrenaline inhibition of whole-cell Ca^{2+} -channel currents in sympathetic neurons. **a, b**, Effect of 3 μM NA in the absence and presence of 10 μM yohimbine. Yohimbine block was reversible (data not shown). **c**, Incomplete inhibition of Ca^{2+} -channel currents at maximally effective NA concentrations. Four records were taken: before (con) and after exposure to 10 μM NA, ~30 s after NA removal (wash), and after exposure to 100 μM NA; 10 μM and 100 μM NA were equally effective. Note reversibility of NA effect with standard internal solution (300 μM GTP). **d**, With 50 μM GTP- γ -S instead of internal GTP, the inhibitory effects of NA were irreversible over 9 min of washing in drug-free solution. **e–g**, Recordings of Ca^{2+} -channel currents evoked by step depolarizations to -20 mV from different holding potentials in the absence and presence of NA (100 μM). Same cell as in **c**, 2 mM external Ba^{2+} . The dependence of the NA effect on holding potential is consistent with inhibition of N-type Ca^{2+} -channel current. Under these recording conditions, L-type channels contribute a relatively sustained current, whereas N-type channels show a greater but not complete degree of inactivation with depolarization^{6,25-27}. In other experiments with 2–10 mM external Ca^{2+} , NA had little or no effect on sustained currents (presumably L-type channel current) evoked by depolarization from -40 or -30 mV (ref. 37).

METHODS. Neurons were isolated from sympathetic ganglia of adult frogs by a combination of enzymatic and mechanical dissociation (see, for example, ref. 24). Currents carried by voltage-gated Ca^{2+} channels were recorded with the whole-cell patch-clamp method. To minimize Ca^{2+} -dependent inactivation and to block residual outward K^{+} currents, Ba^{2+} was usually used as the permeant divalent cation but similar results have been obtained with 2–10 mM Ca^{2+} . The standard internal (pipette) solution contained (mM): CsCl (100), EGTA (10), Na-ATP (2), MgCl (5), GTP (0.3), HEPES (40) (pH 7.2 with CsOH). Recording pipettes had resistances <1 M Ω . The external bathing solution contained (mM): tetraethylammonium (TEA) (130), CsCl (5), Glucose (10), BaCl_2 (2), tetrodotoxin (TTX) (1 μM), propranolol (10 μM) and HEPES (5, pH 7.4 with TEA-OH). Cells were continually superfused at 1–2 ml min⁻¹ with NA-free or NA-containing solutions. A computer (PDP 11/23) controlled the command voltage, and digitized and stored the filtered current signals (-3 dB at 1 kHz). Step depolarizations lasting 320 ms were applied every 10–12 s. All current records were leak-subtracted and are shown together with the voltage protocol. All experiments were carried out at room temperature (~22 °C).

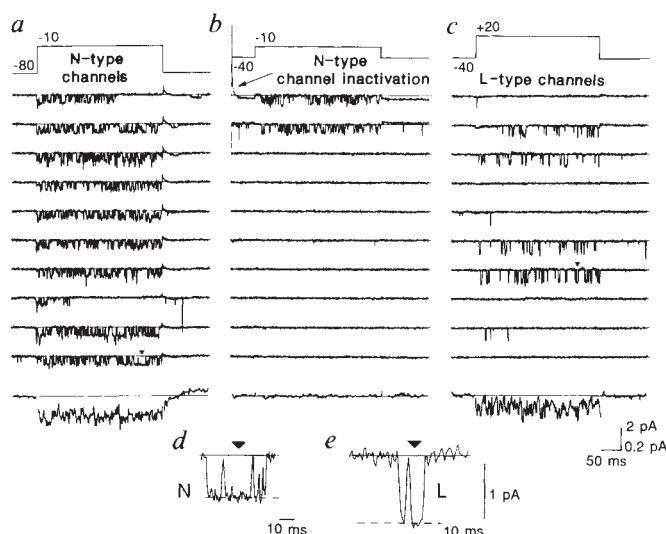


FIG. 3 *a-e*, Separation of unitary N-type and L-type Ca^{2+} -channel currents in the same cell-attached patch. *a*, Sequential recordings of N-type Ca^{2+} -channel currents evoked every 10 s by 320 ms pulses from -80 mV to -10 mV. *b*, Sequential current recordings showing N-type Ca^{2+} channels inactivating within 10–20 seconds following a displacement of the holding potential from -80 to -40 mV (arrow marks corresponding capacitive transient). *c*, Sequential recordings of L-type Ca^{2+} -channel currents evoked by pulses from -40 mV to +20 mV. Average currents, each calculated from ~30 sweeps, are shown below individual current records. *d, e* Openings of N- and L-type Ca^{2+} channels (arrow heads in *a, c*) enlarged to show clearly resolved and different unitary amplitudes. As the unitary L-type currents at +20 mV (*e*) are larger in amplitude than unitary N-type currents at -10 mV (*d*), despite the smaller driving force for Ba^{2+} entry, they must represent different Ba^{2+} conductances (N-type, 15–16 pS; L-type, 26–28 pS; refs 24, 27). *f-h*, Mean currents from separate groups of cell-attached patches, showing inhibition of N-type Ca^{2+} -channel current evoked by 130 ms pulses from -80 mV to -10 mV. Channel activity was identified as N-type by unitary conductance and sensitivity to holding potential as in *a, b*. Averages weighted individual patches equally. *f*, Control ($n=22$ patches); *g, h*, 30 μM NA ($n=10$) or 100 μM NA ($n=5$) in the patch pipette. Noradrenaline (10 μM) reduced N-type channel currents only slightly; a difference in NA-sensitivity between recordings with 110 mM external Ba^{2+} (*f-h*) and 2 mM Ba^{2+} (Fig. 2) might be expected from alterations in surface potential and local catecholamine concentration. *i, j*, Evidence against involvement of a readily diffusible messenger. In five cell-attached patches (pipettes ~1 μm in diameter), the mean current through N-type channels remained unchanged following application of 100 μM NA to the rest of the cell. In none of the experiments was there a detectable decrease in activity after the drug addition.

METHODS. Unitary currents recorded in a series of cell-attached patches with and without NA present in the patch pipette. Attempts at perfusing the pipette with drug while continuously monitoring channel activity were hampered by instability of the recordings, and outside-out patches gave inconsistent results. Recording pipettes had resistances of 5–10 M Ω and contained (mM): BaCl_2 (110), HEPES (10), (pH adjusted to 7.4 with TEA-OH) and TTX (1 μM). The external bathing solution, used to set the membrane potential to zero, contained (mM): K-aspartate (140), Glucose (10), HEPES (5), EGTA (10) (pH adjusted to 7.4 with KOH). A computer (PDP 11/23) controlled the command voltage; and digitized and stored the filtered currents (-3 dB at 1 kHz). Individual current recordings were leak-subtracted before determination of average currents.

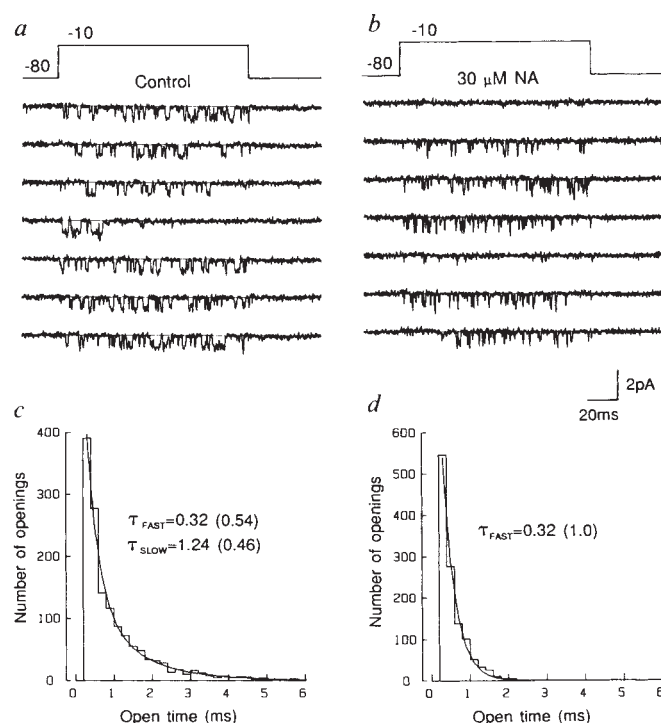


FIG. 4 Effect of NA on the gating of unitary N-type Ca^{2+} channels. *a, b*, Sequential sweeps of N-type Ca^{2+} channel currents recorded in two cell-attached patches, in the absence of NA (*a*) and with 30 μM NA present in the pipette solution. (*b*). Noradrenaline altered gating kinetics, but did not change unitary current size: control, 0.92 ± 0.02 pA ($n=22$); 30 μM NA, 0.95 ± 0.05 pA ($n=8$); 100 μM NA, 1.0 ± 0.02 pA ($n=5$). *c, d*, Histograms of open time durations measured from the patches illustrated in *a, b*. Opening and closing events were detected as crossings of a threshold set halfway between open and closed levels. The distribution of open times in the absence of NA (*c*) was fitted by least-squares with the sum of two exponential components of the form $B \exp(-t/\tau)$ with time constants and relative area $[B \cdot \tau / \Sigma(B \cdot \tau)]$ as indicated. In the presence of 30 μM NA, the slow component was essentially absent (*d*), consistent with the diminished appearance of long openings in the current records (*b*). In other experiments, single channel analysis was performed on records with little or no overlap of unitary currents, like those illustrated in *a, b*. The slow component was too small to measure in 6 out of 12 patches with 30–100 μM NA. In the other 6 patches, a slow component remained detectable, but it decayed more rapidly and was smaller in amplitude and relative area.

been reported, it will be interesting to see if this pattern of modulation of rapid gating kinetics holds true for other neurotransmitters and other cells^{24,26}. Changes in the kinetics of opening and closing might be consistent with allowed voltage-dependence of gating reported by Bean¹⁹.

The pharmacological properties of NA inhibition of whole-cell N-type channel current and transmitter release are very similar. This points to a functional relationship between these phenomena and reinforces earlier evidence that dihydropyridine-insensitive N-type Ca^{2+} channels are the main Ca^{2+} entry mechanism controlling transmitter release from sympathetic neurons⁶. Interestingly, the triggering of transmitter release may be dominated by L-type channels in certain other neuronal systems³⁰⁻³². Stimulation of α -adrenergic receptors is coupled to inhibition of N-type Ca^{2+} -channels and reduction

of transmitter release by means of a G protein but not by a readily diffusible second messenger such as cAMP (as in current hypotheses²⁻⁴), nor by protein kinase C (as in sensory neurons³³). A relatively direct coupling mechanism would be appropriate for rapid feedback control of transmitter release. The feedback may also be localized, because (1) the concentration of NA falls off steeply with increasing distance from the release sites, (2) the α -adrenergic modulation of N-type Ca^{2+} channels works only at short range, and (3) the attenuation of Ca^{2+} entry may strongly affect only nearby release sites. Our results do not exclude additional effects of NA on potassium channels^{34,35}, possibly mediated by clonidine-sensitive α_2 -receptors³⁴ and lowered cAMP³⁵, that would result in less localized decreases in transmitter release through reduction of action-potential duration and global Ca^{2+} entry. □

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A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning

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VOLTAGE-activated potassium channels play an important part in the control of excitability in nerve and muscle. Different K^+ channels are involved in establishing the resting potential, determining the duration of action potentials, modulation of transmitter release, and in rhythmic firing patterns and delayed excitation¹. Using *in vitro* transcripts made from a directional complementary DNA library we have isolated, by expression cloning in *Xenopus* oocytes, a novel K^+ -channel gene (*drk1*). Functionally, *drk1* encodes channels that are K^+ selective and belong to the delayed rectifier class of channels, rather than the A-type class encoded by the *Shaker* gene of *Drosophila*. The channels show sigmoidal voltage-dependent activation and do not inactivate within 500 ms. Structurally, *drk1* encodes an amino-acid sequence which is more closely related to the *Drosophila* *Shab* gene than to the *Shaker* gene.

Several cDNA clones encoding K^+ channels have been isolated from *Drosophila*²⁻⁸. Microinjection of *Xenopus* oocytes, with RNA transcribed *in vitro* from some of the clones produces voltage-dependent, fast transient outward K^+ currents, characteristic of A-type channels⁹⁻¹¹. The different cDNA clones that

have been isolated contain an identical core region, yet differ in the regions encoding the amino and carboxyl termini of the proteins. Using *Shaker* sequence information, two mammalian K^+ -channel cDNA clones encoding the same gene were recently isolated by cross-hybridization^{12,13}. Expression of rat cDNAs in *Xenopus* oocytes yielded K^+ currents with properties of delayed rectifier channels¹⁴ and possibly A-type channels¹⁵. We designed a sequence-independent approach to isolate cDNA clones encoding channel and receptor genes expressed in the brain. Size-fractionated rat-brain messenger RNA, enriched for mRNA between 3.3 and 4.2 kilobases (kb), was used to generate a directional cDNA library in the transcription-competent vector λZAP^{16} . Pools of 100,000 recombinant phages (independent cloning events) were amplified and used to prepare DNA templates for RNA synthesis. Following microinjection of *in vitro* synthesized RNA, *Xenopus* oocytes were tested for outward currents (I_K) produced by depolarizing voltage steps. Transcripts from pools of 100,000 recombinants yielded small I_K -like currents. One pool was divided into smaller 'cocktails', each containing 10,000 recombinants. Three of seven such cocktails elicited sustained outward currents of several hundred nA in amplitude upon depolarization of the oocyte membrane. The currents activated relatively slowly (>100 ms for full activation) and did not inactivate during the test pulse (500 ms). One cocktail was chosen and, by reducing the pool size to 1,000, then 100 and finally 12 recombinants, we eventually isolated a single 'positive' clone (*drk1*) with a 3.4-kb insert.

The cDNA clone we isolated encoded a K^+ channel with the properties of a delayed rectifier¹. The channels opened at test potentials more positive than -20 mV and showed sigmoidal voltage-dependent activation (Fig. 1a, b). The time to half-maximal activation ranged from 20 to 100 ms. Injection of as little as 20 pg *in vitro*-synthesized transcripts produced I_K -current amplitudes of up to 1 μA at +40 mV. At higher current

densities, resulting from injection of 2 ng RNA, it was possible to record from macropatches containing several hundred channels, where the changes in potential are complete in much less than 1 ms (manuscript in preparation). Voltage dependence and kinetics were similar for both macropatch and whole-cell recording. To characterize the ionic selectivity of these channels we determined the reversal potential from tail-current measurements (Fig. 1c). Substituting external Na^+ with *N*-methyl-D-glucamine $^+$ (NMDG) had a negligible effect, whereas varying the external K^+ concentration ($[\text{K}^+]$) shifted the reversal potential with a slope of 48 mV per 10-fold change in external $[\text{K}^+]$ (Fig. 1d), which is characteristic of channels selective for K^+ ions over Na^+ ions. These K^+ currents were blocked by 4-aminopyridine with a 50% inhibitory concentration (IC_{50}) of 0.5 mM and by tetraethylammonium with an IC_{50} of 10 mM (data not shown). The outward current at +20 mV was independent of the presence of Ca^{2+} in the bath and insensitive to Co^{2+} (2 mM) or Cd^{2+} (0.2 mM), eliminating the possibility of the presence of Ca^{2+} -activated K^+ channels. Neither apamin nor charybdotoxin at concentrations up to 1 μM had any effect on the channels.

The DNA sequence of *drk1* was determined and shows an open reading frame of 2,559 nucleotides (Fig. 2a) encoding a protein of 853 amino acids (with a calculated relative molecular

mass of 95,294). The 5'-untranslated region contains 13 nucleotides (the first 8 nucleotides shown in Fig. 2a are linker-derived) and no upstream ATG preceding the assigned initiation codon (that is, the first ATG found). The TGA stop codon is followed by a 3'-untranslated region of ~800 nucleotides ending in a poly(A) tail of ~50 residues. The beginning of the derived protein sequence contains hydrophilic and charged amino acids and presumably does not represent a signal peptide. A hydrophathy plot of the deduced amino-acid sequence shows features reminiscent of all voltage-gated ion channels cloned so far (Fig. 2b). In the N-terminal half of the molecule, there are six hydrophobic regions that have been attributed to membrane spanning segments (boxed regions Fig. 2b). The other half of the protein encoded by *drk1* does not show main hydrophobic regions and probably represents a cytoplasmic tail of >400 amino-acid residues. It is longer than the corresponding C-termini of all other K^+ channels known and it does not show similarity to any known sequence in the protein-sequence data bank. This part of the *drk1* product contains two consensus sequences for cyclic AMP-dependent phosphorylation 17 . The tail may be important in modulation of channel activity and/or targeting of the channel to specific subcellular compartments.

The N-terminal half of the amino-acid sequence derived from the *drk1* clone is similar to the core regions of K^+ channels of

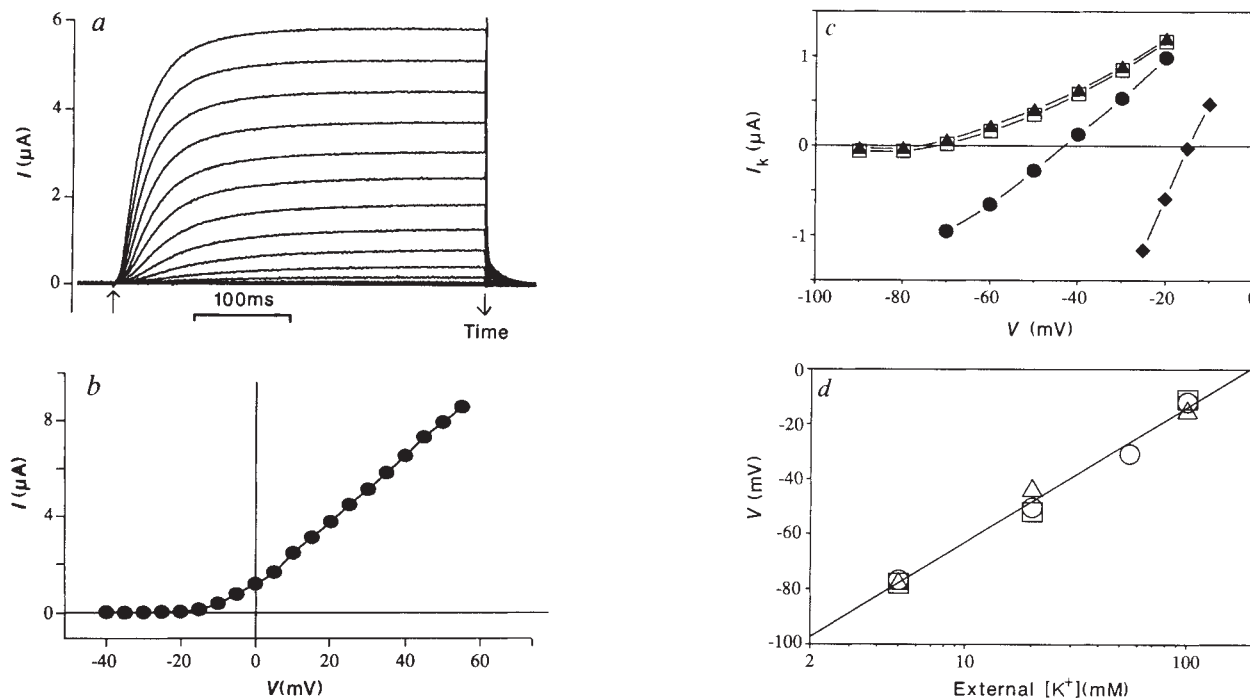


FIG. 1 Delayed rectifying kinetics and K^+ selectivity of *drk1* mRNA expressed in *Xenopus* oocytes. *a*, Family of K^+ currents elicited by 400 ms voltage steps from a holding potential of -50 mV to test potentials ranging from -40 mV to +30 mV in 5-mV increments. The beginning and the end of the pulse are indicated by arrows. Leakage and capacity currents are corrected for by adding the response to a voltage step of equal size but opposite sign. *b*, Steady-state current-voltage relationship for the experiment shown in *a*. The current at the end of the 400-ms test pulse is plotted against membrane potential. Threshold for activation was -15 mV. *c*, The effect of varying external K^+ and Na^+ concentrations on the instantaneous current-voltage relationship. The K^+ conductance was activated by a 200-ms voltage step to 0 mV and repolarized to potentials between -10 mV and -100 mV in 10-mV increments. The instantaneous current following the repolarization is estimated by fitting a double-exponential function to the tail-currents which is extrapolated back to the time of the step. The resulting instantaneous currents are corrected for a linear leakage component estimated from the steady-state currents between -50 and -100 mV and plotted against voltage. The external solutions contained MgCl_2 (2 mM), CoCl_2 (2 mM), HEPES (10 mM, pH 7.4) to which was added (mM): NaCl (100), KCl (5) ($\square$), NMDG (100), KCl (5) ($\blacktriangle$), NMDG (85), KCl (20) ($\bullet$), NMDG (5), KCl (100) ($\blacklozenge$). NMDG was used to replace Na^+ . *d*, Reversal potential as a function

of external K^+ concentration. Reversal potentials were measured from instantaneous current-voltage relationships as shown in *c*. Results from three oocytes are combined. A linear least-squares fit is shown with a slope of 48 mV per 10-fold change in external $[\text{K}^+]$.

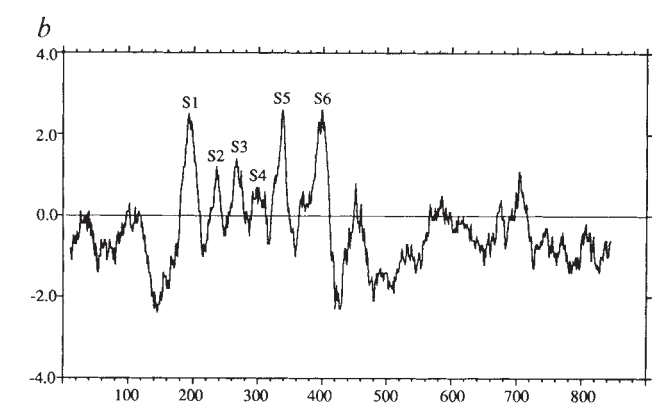
METHODS. A directional rat-brain cDNA library enriched for full-length inserts between 3.3–4.2 kb 16 was used for *in vitro* transcription of sense-strand RNA using T7 RNA polymerase and the 5'-cap analogue m 7 G(5')ppp(5')G (ref. 19). After microinjection of RNA (5 ng), *Xenopus* oocytes were screened 3–4 days later for the expression of delayed rectifying K^+ channels using a two-electrode voltage-clamp technique (ref. 20, and R.H.J. *et al.*, manuscript in preparation). A 'positive' pool of 100,000 recombinants was subdivided into pools of progressively smaller sizes and eventually led to the isolation of the clone *drk1*. Oocytes were isolated from *Xenopus* frogs as described elsewhere (ref. 20, and R.H.J. *et al.*, manuscript in preparation) and kept in modified Barth's solution at 19 °C. For screening they were transferred to a recording chamber and kept in a solution containing (mM): NaCl (140), KCl (2), CaCl_2 (2), MgCl_2 (2), HEPES (10, pH 7.4). Electrodes were filled with KCl (2 M), HEPES (10 mM, pH 7.2). Their resistance in the bath ranged from 1 to 3 M Ω . Oocytes were voltage-clamped using an Axoclamp 2A (Axon Instruments).

1	MetThrLysHisGlySerArgSerThrThrSerSerLeuPro	434	ArgAlaLysArgAsnGlySerIleValSerMetAsnMetLysAspAlaPheAlaArgSer
-21	AATTCGCGTCGCTGGCTGGCATGACGAAGCATGGCTCGCGCTCCACAGCGCTCGTGGCG	1300	AGAGCCAAAGAGGAATGGCAGCATCGTGTCCATGAACATGAAGACGCCGCTCGCCCGGAGG
14	ProGluProMetGluIleValArgSerLysAlaCysSerArgArgValArgLeuAsnVal	454	IleGluMetMetAspIleValValGluLysAsnGlyGluSerIleAlaLysLysAspLys
40	CCCGAGGCCATGGAGATCTGTCGCCGAGCAGCGGTGCTCGCGCGGGTGGCGCTCAACGTC	1360	ATCAGATGATGGACATCTGCTGGGAGAAAAATGGCGAGAGCATAGTAAAGAAGATAAA
34	GlyGlyLeuAlaHisGluValMetLeuTrpArgThrLeuAspArgLeuProArgThrGlu	474	ValGlnAspAsnHisLeuSerProAsnLysTrpLysTrpThrGluAlaLeuSerGlu
100	GGGGGGCTGGCGGACGAGGTGCTGTGGCGCATCTGGACCGCTGCCCGACCGGGGCTG	1420	GTGCAAGATGAACACCTGTCTCCCAACAGTGGAAATGGACAAGAGGCGCATCTCCGAG
54	GlyLysLeuArgAspCysAsnThrHisAspSerLeuLeuGlnValCysAspAspTyrSer	494	ThrSerSerSerLysSerPheGluThrLysGluGlnGlySerProGluLysAlaArgSer
160	GGCAAGCTTCGGGACGTCAACAGCGCAGCATCTCCTGTCTCAGGTGTGGCAGCATTACAGC	1480	ACCAGCTTCAGTAACTCCTTTGAACCAAGGAGCAGGATCCCCGAAAAGCGCAGGCTCC
74	LeuGluAspAsnGluTyrPhePheArgArgHisProGlyAlaLeuSerIleLeuAsn	514	SerSerSerSerProGlnHisIleAsnValGlnGlnLeuGluAspMetTyrSerLysMetAla
220	CTTGAGGACAGCAGTACTTCTTGACCGCACCTGGCGGCTTTACTCTCATCTCAAT	1540	TCGCTAGTCCACAGCATTTGAACGTCACAGCATGGAAGCATCTACAGTAAAGTGGCC
94	PheTyrArgThrGlyArgLeuHisMetMetGluGluMetCysAlaLeuSerPheSerGln	534	LysThrGlnSerGlnProLeuMetAsnThrLysGluMetAlaProGlnSerLysProPro
280	TTCTACCGCACCGCGCGGTGCATCATGATGAGGAGATGTGCGCGGTGAGCTTACGCCAG	1600	AAGACACAGTCCCGCCCTCTCTCAACACCAAGGAGATGCCCGCGAGACAGGCTCCA
114	GluLeuAspTyrTrpGlyIleAspGluIleTyrLeuGluSerCysGlnAlaArgTyr	554	GluGluGluMetMetSerSerMetProSerProValAlaProLeuProAlaArgThrGlu
140	GAGCTGGACTACTGGGCGACGTGATGAGATCTACCTGGAGTCTCTGCTGCCAGGCCGCTAT	1660	GAGGAGCTGGAGATGAGCAGCATGCCACGCCCTGGCCCTCTGCCCGCACGACGGAG
134	HisGlnLysGlyGluValMetAsnGluGluLeuLysArgGluAlaLeuIleSerIleMet	574	GlyValIleAspMetArgSerMetSerSerIleAspSerPheIleSerCysAlaThrAsp
400	CACCAAAGAAGGACAGATGAACAGGAGCTGAAGCGCGAGGCCGAGACGCTGCGGGAG	1720	GGCTCTACGCATCGGAGCATGTCCAGCATTGACAGCTTCAGTCTGTGCCACGGAC
154	ArgGluGlyGluGluPheAspAsnThrCysCysAlaGluLysArgLysLysLeuTrpAsp	594	PheProGluAlaThrArgPheSerHisSerProLeuAlaSerLeuSerSerLysAlaGly
460	CGGGAGGGCGAGGAGTTTGACAACACGCTGCTGTGCAGAGAGAGGAAGAATCTGGGAT	1780	TTCCCTGAAGCCACAGATCTCCCAAGTCTCTCGGATCTCCTCTCCAGCAGAGGCTGG
174	LeuLeuGluLysProAsnSerArgValAlaAlaLysIleAlaLeuIleSerIleMet	614	SerSerSerGluGluProValGlyTrpArgGluLeuAlaGlyIleAlaSerGlyGlyArgLeu
520	CTGCTGGAGAAGCCCAACTCGTCGGTCTCCCGCAAGATCTGGCCATCATCTCGATCATG	1840	AGCAGCACAGCCCGAGGTGGGCTGGCGGGGGCTCTGGGTGCAGCAGCGGGAGGCTC
194	PheIleValLeuSerThrIleAlaLeuSerLeuAsnThrLeuProGluLeuGlnSerLeu	634	ThrGluThrAsnProIleProGluThrSerArgSerGlyPhePheValGluSerProArg
580	TTTCATCGCTCTGCTCCACCATCGCTCTGTCATCTACACGCTGCTCGAGCTGCAGAGCCTA	1900	ACGGAGACCAACCCCATCTCTGAGACAGCGCGCTCTGTGTTTCTCTGGAGAGCTCCGAG
214	AspGluPheGlyGlnSerThrAspAsnProGluLeuAlaHisValGluAlaValAlaGly	654	SerSerMetLysThrAsnAsnProLeuLysLeuArgAlaLeuLysValAsnPheValGlu
640	GACGATTCGGCGACGACAGGATAACCCGACCTGGCGCCAGCTGGAGGGCGTGTGCATC	1960	AGTTCTCATGAAGACCAACAACCTCTGAGCTGCGAGCGCTCAAGGTCAACTCTGTGGAG
234	AlaTrpPheThrMetGluTyrLeuLeuArgPheLeuSerSerProLysLysTrpLysPhe	674	GlyAspProThrProLeuLeuProSerLeuGlyLeuTyrHisAspProLeuArgAsnArg
700	GCATGGTTCACCATGGAGTACTTGTGAGGTCTCTGTCTCTCCCAAGAAATGGAAGTTC	2020	GGGACCCCAACCCCGCTGCTGCCCTCCCTGGCTGTGATACAGATCTCTTTAGAACAGA
254	PheLysGlyProLeuAsnAlaIleLeuLeuAlaIleLeuProTyrValThrIle	694	GlyGlyGluAlaAlaValAlaValAlaGlyLeuGluCysAlaSerLeuLysAspLysProVal
760	TTCAAGGGCTCCCTCAACGCCATTGACCTATGGCCCATCTGCCCTACTACGTCAACCATC	2080	GGAGGGGACAGCGCTGCACTTGGCGGATGAGTGTGCTCCCTCTTAGACAGGAGCTG
274	PheLeuThrGluSerLysLysSerValLeuGlnPheGlnAsnValArgArgValValGln	714	LeuSerProGluSerSerIleTyrThrThrAlaSerAlaArgThrProProArgSerPro
820	TTCTCTCACAGATCTACACAGAGCGTGCTGCAGTTCACAGACCTGGCGCGGTGGTCCAG	2140	CTGAGCCCAAGATCTCTCATCTACACACAGCAAGTGCAGGACGCGCCCTCGTCCCTCT
294	IlePheAlaIleMetArgIleLeuArgIleLeuLysLeuAlaArgHisSerThrGlyLeu	734	GlyLysHisThrAlaIleAlaPheAsnPheGluAlaGlyValHisHisTyrIleAspThr
880	ATCTTTTCGATAGTGGCATCTCTCGGATCTGAAGCTGGCCGCTACTCCATCCATGGCTG	2200	GAGAAGACACAGCATAGCATCTCAACTTGGAGCGGAGTCCACCATCATAGACACC
314	GlnSerLeuGlyPheThrLeuArgArgSerTyrAsnGluLeuGlyLeuLeuIleLeuPhe	754	AspThrAspAspGluGlyGlnLeuLeuTyrSerValAspSerSerProProLysSerLeu
940	CAGTCTCTGGGCTTCACCTGGCGCAGAGCTACAACCATCTGGGCTGCTCATCTCTTCT	2260	GACACAGATGACAGGGGTGACGTGCTCTACAGCGTGGATCCAGCCCTCCCAAAAGTCTCT
1000	CTCGCATGGGATCATGATCTTCTCCAGCGTGGTCTTCTCGCGCAAGGATGAGGAC	774	HisSerGlyThrSerProLysPheSerThrGlyAlaArgThrGluLysAsnHisPheGlu
354	AspThrLysPheLysSerIleProAlaSerPheTrpTrpAlaThrIleThrMetThrThr	794	SerSerProLeuProThrSerProProLysPheLeuArgProAsnCysValTyrSerSerGlu
1060	GACACCAAGTTCAAAGCATCCCGGCTCTTCTGGTGGGTACCATCACCATTGACAAC	2380	AGTCTCCCTTGGCCACTCCCTTAAGTCTTAAAGCCGAACTGTGCTACTCTCAGAA
1120	ValGlyTyrGlyAspIleTyrGlyLysThrLysLeuLysLysIleValGlyGluGluGlu	814	Gly
1180	GTGGCTATGGAGACATCTACCCAGACATCTCTGGGAAATCTGGGGGGGCTCTGT	2440	GGGTTGATCTGAAAAGGCTCTGGGGCTCAAGGAAGTCAAGCTGGAGAACCATTACCCC
394	CysIleAlaGlyValLeuValIleAlaLeuProIleProIleIleValAsnAsnPheSer	834	ProAspValHisMetLeuGlyGlyGlyAlaHisGlySerThrArgAspGlnSerIle
1180	TGCAATGCCGGGCTCTGGTGATTGCTCTGCCATCCCATCATCGTCAATTAATCTCTCT	2500	CCGGATGTGCACATCTGCTGGGGGAGGACGACGAGGAGCTAGGATCAGAGTATC
414	GluPheTyrLysGluGlnLysArgGlnGluLysAlaLysArgArgGluAlaLeuGlu	2560	TGAGTCCCTTACCACAGAGGGCATCTGCA-.....(approx 800 nucleotides).....3'end
1240	GAGTCTCTCAAGGACGAGGAGCGGAGGAAAGCATCAAGCGGAGGAGGCTCTGGAG		

FIG. 2. *a*, Nucleotide- and deduced amino-acid sequence of *drk1*. The numbers indicate amino-acid and nucleotide positions starting at the designated initiation codon. To obtain the longest possible reading frame, the first ATG triplet was chosen as the initiation codon. The sequence flanking the second in-frame ATG (position 49–51), however, is in better agreement with the consensus sequence for translation initiation²¹. Amino acids 77–83 (heavy line on top) are conserved in all K⁺ channels cloned so far. The boxes outline potential transmembrane segments S1–S6. Consensus sequences for *N*-glycosylation²² are shown by asterisks. Only one site (position 279) faces the extracellular side in the assumed topology of the channel. Two consensus sequences for cAMP-dependent phosphorylation (●) are found in the cytoplasmic tail¹⁷. *b*, Hydropathy plot of the amino-acid sequence of *drk1*. The deduced amino-acid sequence of *drk1* was subjected to a hydropathy analysis according to Kyte and Doolittle, with a window size of 22 amino acids²⁵. Segments S1–S6 correspond to the boxed regions in Fig. 2. Amino-acid positions are shown on the abscissa. The relative hydrophobicity index is shown on the ordinate.

METHODS. The DNA sequence of *drk1* was determined for both strands of the insert. Several restriction fragments were subcloned into M13mp19

Drosophila (those encoded by *Shaker*)⁶⁻⁸, rat and mouse¹²⁻¹⁵, and to the core regions of two different putative K⁺ channels encoded by the genes *Shab* and *Shaw*¹⁸. Figure 3 shows an alignment of the *drk1* sequence (corresponding to residues 18-430) with sequences of the *Drosophila* genes *Shaker*, *Shab*, and *Shaw* and with one of rat brain (*rc1*). In this core region, the percentage of identical amino acids in analogous positions between channels is given in Table 1. The six putative membrane-



vectors using standard recombinant DNA technology and the sequences were determined using an ABI automated DNA sequencer^{23,24}.

spanning segments (S1–S6) and a stretch of seven amino acids (Asn-Glu-Tyr-Phe-Phe-Asp-Arg) preceding S1 show the highest conservation within the core region of the five channels analysed. The segment S4 is probably the voltage-sensor and is characterized by a series of positively charged amino acids at every third position. The S4s of the *Shab* and *drk1* products have five positive charges; those of the *rck1* and *Shaker* products contain two additional positive flanking charges. Only four such charges

<i>drk1</i>	EIVRSKACSRRLNVGLLAHEVLWRTLDRLPRLGLKRLDCNTHDSLQVCDYSLDNEYFFDRHPGA	87
<i>shab</i>	M-AQ---VNS--SI---VR---E---H---R-GE-T-EAIVEL-----A-----KS	337
<i>rck1</i>	ADHDDHE-CE--VI-IS--RF-TQLK--AQF-N-L-NPKKRMRY	88
<i>sh</i>	P-PHDHDFCE--VI-S--RF-TQL--NQF-D-L-DPARRLY	148
<i>shaw</i>	MNLINMDSN--V-----IR--TYKA--KKI-A--SR-TEALAN	61
<i>drk1</i>	FTSILNFYRTG RLHMEEMCALSFQELDYWGIDEIYELSCQARYHQKKEQMNEELKREAETLREREG	156
<i>shab</i>	-S-----K-IVD---V-A-GDD-E---V-L-----HK---R-NVH-MRK---S-Q-DE	406
<i>rck1</i>	-DA--YY-QS-G--RRPVNPLDM--E-IFKYLGL-EAM-KFREDEGFI-E-ERPL	144
<i>sh</i>	-DA--YY-QS-G--RRPVNPLDV--E-IFKYLGLDQAIKFREDEGFI-E-ERPL	204
<i>shaw</i>	-AQV--Y---K--YPTDV-GPL-EE-EF--L-SNQV-P--WMT-T-HRDTQETLAVLDRLD-DTEKP	130
S1		
<i>drk1</i>	EEFDNT	211
<i>shab</i>	---GEG	461
<i>rck1</i>	CCAEKRKKLWDLLEKPNSSVAARILAIISIMFIVLSTIALSINTLPELQ	193
<i>sh</i>	KFS-YQ-Y-E-E---RVV-V--L---IVIFC-E---K	253
<i>shaw</i>	PEK-YQYQV-L-F-Y-E-GP-RVI-V-V-L-I-IVIFC-E---K	200
	PDN--QR-V-L-F-Y-E-Q--RVV--VFV-L-I-IVIFC-E---FK	
	S-EELARKFGFEEDYYKGTISWWQ-MKPRI-S-FDE-Y-N--TIGVV-VF-CI-ILSFC-K-H-DMR	
S2		
<i>drk1</i>	SLDEFGQSTDNPCLAHVEAVCIAWFTMEYLLFLSSPKKWKFKGFLNATDILL	264
<i>shab</i>	HIDN-TPQ---	513
<i>rck1</i>	DDKDFGTGIHRIDNTTIVYTSNIFTDP	260
<i>sh</i>	HYKVFNTTNGTKIEDEVDITDP	318
<i>shaw</i>	VPVIRNITVKTANGSNGWFLDKT-TNAHIAFFYI-C-N---F-T-V-I-N-E-I-SSV-I-YI	270
S3		
<i>drk1</i>	AILPYYVTIFLTESNKSVL	319
<i>shab</i>	---F-SL-L-T--NATD	569
<i>rck1</i>	---I--FI-LGTEIAEQEGNKG	318
<i>sh</i>	---I--FI-LATVVEEDTLNLFKAPVSPQKSSNQAMSLAILLV-LV-VF--F-S--M--I--R	388
<i>shaw</i>	-T-SF-IDLV-ORHA	321
S4		
<i>drk1</i>	QFQNVRRVHVQIRIMRIILRIILKLAHSTGLQSLGFT	319
<i>shab</i>	---D-SL-L-T--NATD	569
<i>rck1</i>	EQATSLAILLV-LV-VF--F-S--M--I--R	318
<i>sh</i>	---I--FI-LATVVEEDTLNLFKAPVSPQKSSNQAMSLAILLV-LV-VF--F-S--M--I--R	388
<i>shaw</i>	SHLENAHILF-S-N-M-LF-VT--S--KI-IQ-	321
S5		
<i>drk1</i>	LRRSYNELGLLILFLAMGIMIFSSLVFFAEKDEDDTK	387
<i>shab</i>	---N-K--M--VL---AY--K--	637
<i>rck1</i>	-KA-MR---F--FI-VIL--A-Y--AE-AESH	386
<i>sh</i>	-KA-MR---F--FI-VVL--A-Y--AGSENF	456
<i>shaw</i>	F-A-AK--T--VF--VL--V--A--YY--RIQPNPHND-N--LGL--LV-----MA--YI-M	391
S6		
<i>drk1</i>	IVGGLCCIAGVLVIALPIIIVNFSEFYKEQKRQEKAKRRE	430 (+423)
<i>shab</i>	VI-TV---C---V-----A---N-M-R--L---	680 (+244)
<i>rck1</i>	---S-A---T---V-V-S--NY--HRETEG-EQAQLLH	429 (+66)
<i>sh</i>	---S-VV---T---V-V-S--NY--HREADR-EMQSQNF	499 (+117)
<i>shaw</i>	F--A--AL---T---V-V-S--AMY-SHTQARA-LP-K-R	434 (+64)

are present in S4 of the protein encoded by *Shaw*; this is the only (putative) K⁺ channel known to have a negatively charged amino acid (glutamate) at a position where other K⁺ channels have a positively charged arginine residue. It will be interesting to see whether these charge differences are responsible for some of the different kinetic properties of A-type and delayed rectifier channels. From the comparative alignment shown in Fig. 3 and Table 1 it is clear, on the basis of the conserved core sequences, that *drk1* and *Shab* are two members of the same family of genes, and *rck1* and *Shaker* are members of another family. The *drk1/Shab*-family and *rck1/Shaker* family are related and belong to a superfamily which includes a third family with *Shaw* as its only member so far. Of the *drk1/Shab* family only *drk1* has been expressed (this report), and we have shown that it encodes a K⁺ channel with properties of a delayed rectifier. Of the *rck1/Shaker* family several members have been expressed. The *Shaker* clones encode A-type channels⁹⁻¹¹, whereas expression of *rck1* yields K⁺ currents with properties of delayed rectifier-type channels¹⁴ and possibly A-type channels¹⁵. The function of *Shaw* is unknown. The fact that *rck1* and *Shaker* genes are more closely related to one another than to *drk1* encoding the new K⁺ channel indicates that the *rck1/Shaker*

and *drk1/Shab* families are evolutionarily older than flies and mammals. The most conserved region in all K⁺ channels sequenced so far is the stretch of seven amino acids near the beginning of the core. Because it is present in all types, this stretch may be a general hallmark of K⁺ channels. This region seems well-suited to initiate a specific search by means of an oligonucleotide-directed screening approach to identify unknown members of the K⁺ channel superfamily. □

TABLE 1 Percentage of identical amino acids in analogous positions between pairs of different K⁺-channel types

	<i>Shab</i>	<i>Shaker</i>	<i>Shaw</i>	<i>rck1</i>
<i>drk1</i> 70	70	40	41	40
<i>rck1</i>	39	74	40	
<i>Shaw</i>	40	42		
<i>Shaker</i>	36			

Percentages were calculated using the alignment in Fig. 3. The results show that *drk1* is most closely related to *Shab*, whereas *rck1* is most similar to *Shaker*.

FIG. 3 Comparison of amino-acid sequences of different K⁺ channels. The sequences of five different K⁺ channels were aligned to reveal similarities over the areas indicated. Amino-acid positions are shown by numbers. The lengths of the missing C-termini are shown in parentheses. Identical amino acids are indicated by dashes; gaps in the sequences, by empty spaces. The *Shaker* gene encodes an A-type K⁺ channel and is derived from the *Shaker*-region of *Drosophila*⁴. Two *Drosophila* cDNA clones, *Shab* and *Shaw*, whose functions are not known, are distinct from the various *Shaker* clones¹⁸. The gene *rck1* was isolated from a rat-brain cDNA library using a *Shaker* sequence as a screening probe¹³. Two families, each containing two members, are apparent. The gene *drk1* is most similar to *Shab*, whereas *rck1* is most similar to *Shaker*. The putative transmembrane regions (S1-S6) are boxed. The positive charges in the postulated voltage-sensor (S4) are shaded, as well as the conserved charged residues in other transmembrane regions. The longest stretch of identical residues present in all the channels is outlined by a heavy line. We used the EuGene software by Molecular Biology Information Resources at the Department of Cell Biology, Baylor College of Medicine, for the sequence alignment.

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Qa-1 restricted recognition of foreign antigen by a $\gamma\delta$ T-cell hybridoma

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DISTINCT T-lymphocyte subsets recognize antigens in conjunction with different classes of major histocompatibility complex (MHC) glycoproteins using the T-cell receptor (TCR), a disulphide-linked heterodimer associated with the CD3 complex on the cell surface¹. In general, class I and class II MHC products provide a context for the recognition of foreign antigens by CD8⁺ and CD4⁺ T cells, respectively². This recognition seems to be largely dependent on $\alpha\beta$ TCR heterodimers^{3,4}, whereas the function of the second $\gamma\delta$ TCR, present on a minor subpopulation of cells, is still unknown⁵. In the mouse, the existence of six cell-surface MHC class I products (K, D, L, Qa-1, Qa-2 and Tla) has been firmly established by serological, biochemical and genetic evidence⁶. So far, only the most polymorphic of them, K, D and L ('classical' class I) have been reported as restriction elements for T-cell recognition of foreign antigens¹. The function of the relatively invariant Qa and Tla molecules remains unknown^{6,7}. We have made a T-helper cell hybridoma clone (DGT3) that recognizes synthetic copolymer poly(Glu⁵⁰Tyr⁵⁰) (GT) in the context of Qa-1 cell surface product, and has a CD4⁺CD8⁺ phenotype. Our studies indicate that DGT3 cells express the $\gamma\delta$ TCR on the cell surface, implicating its role in Qa-1-restricted antigen recognition. This is the first evidence that T cells can recognize foreign antigen in association with self Qa product, confirming that Qa molecules not only topologically, but also functionally, belong to the MHC.

The T-helper hybridoma clone DGT3 was obtained by fusion of poly(Glu⁵⁰Tyr⁵⁰) (GT)-primed lymph node cells from DBA/2 (*H-2^d*) mice with the AKR (*H-2^k*) thymoma BW5147 (legend to Table 1). It reacted to GT in the absence of any additional antigen-presenting cells (APCs) (Table 1). The response was antigen-specific, because DGT3 reacted to neither of two related antigens poly(Glu⁶⁰Ala³⁰Tyr¹⁰) (GAT) and poly(Glu⁶⁰Ala⁴⁰) (GA). Immunofluorescence analysis of the DGT3 clone revealed its origin (Fig. 1a). Strains AKR and DBA/2 carry different allomorphs of the common T-cell marker (Thy-1.1 and Thy-1.2, respectively). As DGT3 cells express Thy-1.2 determinant, they are derived from DBA/2 T cells. Like all mouse T cells, they express only class I MHC, but do not express either CD4 or CD8, suggesting that either the ancestral clone belonged to a minor subpopulation of peripheral T cells, or the expression of accessory molecules was switched off on fusion with BW5147.

As shown in Table 1, the GT specific activation of DGT3 was not inhibited by monoclonal antibodies specific for either MHC class II, 'classical' class I (K, D, L) or accessory molecules (CD4, CD8, LFA-1), although the response of the CD4⁺CD8⁺ hybridoma clone DGT1 was efficiently blocked by the antibodies specific for A^d, CD4 and LFA-1 (ref. 8). The only reagents that abolished the response of DGT3 were antibodies against Qa-1^b molecules. The abrogation of DGT3 response was Qa-1 allomorph specific, because antibodies against Qa-1^a (not expressed by DBA/2 or AKR strains) did not inhibit the response. As a control, anti-Qa-1^b antibodies did not abolish GT specific response of the A^d restricted clone DGT1. They also did not block activation of either DGT3 with concanavalin A (con A) or the CTLL cell line with interleukin-2 (IL-2). This suggests that the mechanism of inhibition by these antibodies is not due to negative signal transmission through Qa-1^b

molecules, but because of interference with the presentation of GT in the context of Qa-1^b by DGT3 cells themselves. This is confirmed by the experimental data displayed in Table 2. Instead of adding free soluble GT into the culture, different APCs were preincubated (pulsed) with the antigen and used to present membrane-bound GT to DGT clones. Detachment of GT from the surface of pulsed cells was prevented by glutaraldehyde fixation. DGT3 cells could be stimulated by GT pulsed APCs carrying Qa-1^b but not by Qa-1^a molecule, whereas their expression of different Tla or classical H-2 allomorphs was irrelevant. Neither of the class II-negative antigen-pulsed T-cell lines could activate clone DGT1, which required the presence of GT/A^d complex (displayed by B-lymphoma line A20).

It has been reported that Qa and Tla molecules are recognized by unrestricted cytotoxic T lymphocytes in the same way as other MHC antigens. Unlike classical MHC products, they are weak transplantation antigens and they have not been shown

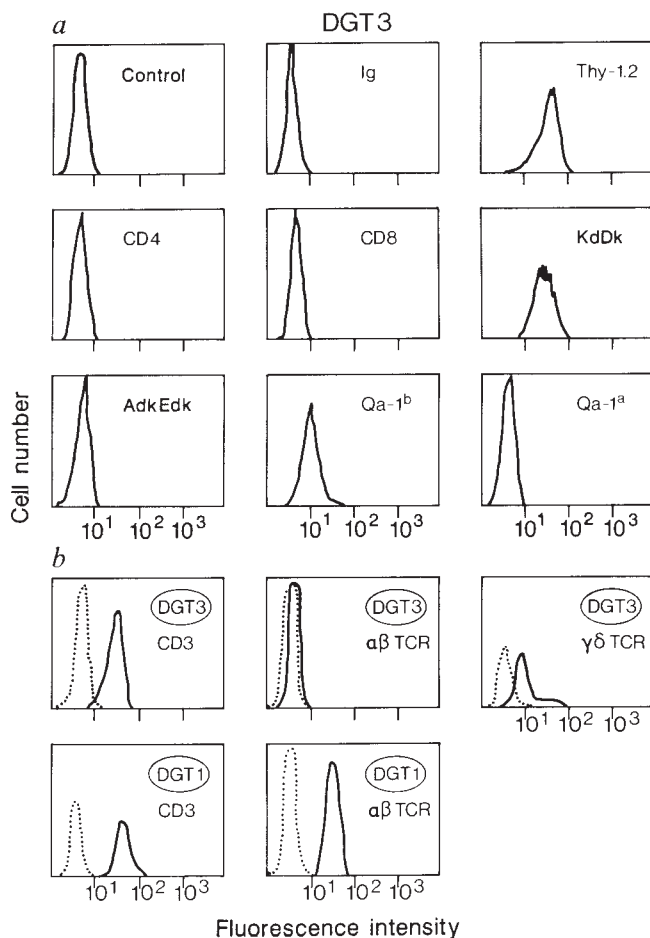


FIG. 1 Cell-surface phenotype of clone DGT3. *a*, T-hybridoma cells DGT3 were stained either directly with fluorescein (FITC) labelled antibodies against mouse immunoglobulin (Ig, Southern), Thy-1.2, CD8 (both from Becton-Dickinson) and CD4 (hybridoma GK1.5; ref. 30) or indirectly by incubation with either anti-K^dD^k (hybridoma 15.5.5S), anti-A^dK^dE^dK^d (hybridoma 82.B) or antibodies against Qa-1 allomorphs (see legend to Table 1), followed by the labelling with FITC-conjugated antibodies specific for mouse immunoglobulin (Southern). 'Control' indicates the cells incubated without addition of antibodies. *b*, DGT3 and DGT1 cells were stained indirectly by incubation with either biotin-coupled anti-CD3 monoclonal antibody (2C11; ref. 31), pan- $\alpha\beta$ TCR reactive monoclonal antibody H57.597 (ref. 12) or pan- $\gamma\delta$ TCR specific monoclonal antibody 3A10 (ref. 13) as indicated, followed by labelling with FITC-conjugated either streptavidin (for CD3; Southern) or goat antibodies specific for hamster or mouse IgG (for $\alpha\beta$ TCR or $\gamma\delta$ TCR, respectively; Southern). Broken lines represent cells stained with second step reagents only. Analysis for *a* and *b* was performed on a FACS (Becton-Dickinson) flow cytometer.

TABLE 1 Antibody blocking of response to GT antigen

Experiment	Responding cells	Antigen	A20	ConA	IL-2	Antibody (specificity)	Response (c.p.m.)
1	DGT3	—	—	—	—	—	6.265
		GT	—	—	—	—	195.089
		GT	—	—	—	15.1.5P (K ^d D ^k)	175.924
		GT	—	—	—	15.5.5S (K ^d D ^k)	199.362
		GT	—	—	—	21.460 (D ^d)	198.036
		GT	—	—	—	28-14-8S (L ^d)	185.596
		GT	—	—	—	polyclonal (Qa-1 ^a)	171.830
		GT	—	—	—	polyclonal (Qa-1 ^b)	42.264
		GT	—	—	—	267-503 (Qa-2 ^a)	172.033
		—	—	+	—	—	191.255
		—	—	+	—	polyclonal (Qa-1 ^b)	185.247
		—	—	—	—	—	3.528*
		—	—	—	+	—	301.097*
		—	—	—	+	polyclonal (Qa-1 ^a)	268.788*
		—	—	—	+	polyclonal (Qa-1 ^b)	281.344*
2	DGT3	—	—	—	—	—	1.002
		GT	—	—	—	—	32.699
		GT	—	—	—	129.19.6.8 (CD4)	41.490
		GT	—	—	—	H35-17.2 (CD8)	29.160
		GT	—	—	—	H68-96.22 (LFA-1)	32.902
		GT	—	—	—	8.A (A ^{d,k})	38.233
		GT	—	—	—	39.I (A ^{d,k} , E ^{d,k})	28.671
		GT	—	—	—	82.B (A ^{d,k} , E ^{d,k})	36.021
		GT	—	—	—	40.I (A ^{d,k} , E ^{d,k})	37.110
		GT	—	—	—	40.B (A ^{d,k} , E ^{d,k})	37.001
		GT	—	—	—	10.A (E ^{d,k})	29.920
		GT	—	—	—	40.D (E ^{d,k})	36.040
		GT	—	—	—	7.A (E ^{d,k})	34.667
		GT	—	—	—	74.C (E ^{d,k})	34.967
3	DGT3	—	—	—	—	—	591
		GAT	—	—	—	—	316
		GA	—	—	—	—	636
		GT	—	—	—	—	22.817
		GT	—	—	—	MK-D6 (A ^d)	22.120
		GT	—	—	—	pool 7 (Qa-1 ^b)	511
		—	—	+	—	—	29.727
		—	—	+	—	pool 7 (Qa-1 ^b)	29.141
4	DGT1	—	—	—	—	—	1.188
		GT	—	—	—	—	1.118
		—	+	—	—	—	1.218
		GT	+	—	—	—	116.608
		GT	+	—	—	MK-D6 (A ^d)	9.041
		GT	+	—	—	polyclonal (Qa-1 ^a)	102.444
		GT	+	—	—	polyclonal (Qa-1 ^b)	110.576
		GT	+	—	—	267-503 (Qa-2 ^a)	112.676
5	BW5147	—	—	—	—	—	1.183
		GT	—	—	—	—	1.214

Hybridoma cells (1×10^5) were cultured with or without APCs (B-cell lymphoma A20, 3×10^4 per well) and antigens. After 24 h the supernatants were collected and tested at 75% concentration for their ability to support the growth of 10^4 CTLL cells (an IL-2 dependent cell line) for 24 h. The mean values for triplicate cultures are shown.

DBA/2 mice were immunized with 100 μg GT (ref. 8). Seven days later, cells from the draining lymph nodes were cultured with 150 $\mu\text{g ml}^{-1}$ GT. Surviving cells were fused with BW5147, a T thymoma whose stimulation can be assayed by the production of IL-2 (refs 21, 22). GT-specific hybridomas were selected with 150 $\mu\text{g ml}^{-1}$ GT, the most effective dose for the population. The resulting hybridomas DGT3 and DGT1 (for DBA/2 GT-specific) were recloned 3 times. DGT1 was Thy-1.2⁺CD4⁺CD8⁺ (ref. 8). The dose of GT, GA and GAT (Sigma) used for the assay was 150 $\mu\text{g ml}^{-1}$. All antibodies were added at the beginning of the culture period. Those specific for Qa-1 allomorphs ('polyclonal') were obtained by protein-A purification from immune antisera²³ and used at final concentration of 5 $\mu\text{g ml}^{-1}$. An independent batch of anti-Qa-1^b antiserum (designated as 'pool 7') was used at final dilution 1:30. The specificity of anti-Qa-1 antiserum was determined by analysing the panel of lymphoid cells from various inbred and congenic mouse strains on a FACScan flow cytometer (Becton-Dickinson) and by inhibition of lympholysis mediated by Qa-1-specific cytotoxic T cells (data not shown). The genetic difference between recipient and donor mice used to generate Qa-1 specific antisera also involves the *Tla* locus. Staining of peripheral mature T cells (that are *Tla* negative), and thymocytes expressing either the third party *Tla* allomorph or no *Tla* product at all, however, excluded possible *Tla* reactivity of those antisera. All monoclonal antibodies directed against class II MHC, CD4, CD8, LFA-1 and D^d (21.460) class I molecules were used at a final concentration of 5 $\mu\text{g ml}^{-1}$. Culture supernatants of hybridomas 15.1.5P and 28.14.8S (ATCC) were diluted twice. Ascitic fluids of the hybridoma antibodies 15.5.5S and 267-503 (Milan Analytica) were used at concentrations of 1%. All monoclonal antibodies were described in refs 12, 24–28. Asterisks indicate direct stimulation of CTL L line with human recombinant IL-2 (Roche).

to restrict the T-cell recognition of other antigens^{6,7}. MHC-restricted recognition of foreign antigens by most of the single positive T cells (CD4⁺CD8[−], CD4[−]CD8⁺) has been definitively attributed to the $\alpha\beta$ TCR (refs 3, 4). The majority of double negative (CD4[−]CD8[−]) peripheral T cells, however, express the $\gamma\delta$ TCR (ref. 5) and it was of interest therefore to characterize

TCR gene usage in the Qa-1^b restricted double-negative DGT3 hybridoma.

Northern blot analysis of DGT3 poly(A)⁺ RNA showed existence of all four TCR gene transcripts (α , β , γ , δ) using constant-region specific complementary DNA probes (Fig. 2a). Mature-sized $\alpha\beta$ TCR messenger RNA (1.5 and 1.3 kilobases

TABLE 2 Stimulation of hybridoma clones by antigen-pulsed APCs.

Hybridoma	Soluble GT	Additional APC		GT-pulsed fixed	Alleles at loci		Antibodies against	Response (c.p.m.)
		non-pulsed	GT-pulsed		<i>Tla</i>	<i>Qa-1</i>		
DGT3	—	—	—	—	—	—	—	5.295
	+	—	—	—	—	—	—	<u>83.799**</u>
	+	—	—	—	—	—	Qa-1 ^a	<u>77.911</u>
	+	—	—	—	—	—	Qa-1 ^b	8.349
	—	—	BW5147	—	<i>b</i>	<i>b</i>	—	<u>52.950</u>
	—	—	BW5147	—	<i>b</i>	<i>b</i>	Qa-1 ^a	<u>43.636</u>
	—	—	BW5147	—	<i>b</i>	<i>b</i>	Qa-1 ^b	7.190
	—	—	EL4	—	(<i>b</i>)*	<i>b</i>	—	<u>58.388</u>
	—	—	EL4	—	(<i>b</i>)	<i>b</i>	Qa-1 ^a	<u>45.864</u>
	—	—	EL4	—	(<i>b</i>)	<i>b</i>	Qa-1 ^b	7.172
	—	—	R1.1	—	<i>a</i>	<i>a</i>	—	5.773
	—	—	R1.1	—	<i>a</i>	<i>a</i>	Qa-1 ^a	5.245
	—	—	R1.1	—	<i>a</i>	<i>a</i>	Qa-1 ^b	6.565
	—	—	—	BW5147	<i>b</i>	<i>b</i>	—	<u>43.092</u>
	—	—	—	EL4	(<i>b</i>)	<i>b</i>	—	<u>38.166</u>
	—	—	—	R1.1	<i>a</i>	<i>a</i>	—	6.388
DGT1	—	—	—	—	—	—	—	3.107
	+	—	—	—	—	—	—	2.838
	—	A20	—	—	<i>c</i>	<i>b</i>	—	4.366
	+	A20	—	—	<i>c</i>	<i>b</i>	—	<u>103.773</u>
	—	—	A20	—	<i>c</i>	<i>b</i>	—	<u>95.364</u>
	—	—	BW5147	—	<i>b</i>	<i>b</i>	—	2.996
	—	—	EL4	—	(<i>b</i>)	<i>b</i>	—	3.066
	—	—	R1.1	—	<i>a</i>	<i>a</i>	—	2.031
	—	—	—	A20	<i>c</i>	<i>b</i>	—	<u>27.572</u>
	—	—	—	BW5147	<i>b</i>	<i>b</i>	—	2.304
	—	—	—	EL4	(<i>b</i>)	<i>b</i>	—	3.096
	—	—	—	R1.1	<i>a</i>	<i>a</i>	—	3.235
DGT3	—	—	—	—	—	—	—	797
	+	—	—	—	—	—	—	<u>72.722</u>
	—	B6*	—	—	(<i>b</i>)	<i>b</i>	—	942
	—	B6-Tla ^a *	—	—	(<i>a</i>)	<i>a</i>	—	1.005
	—	BALB/c*	—	—	(<i>c</i>)	<i>b</i>	—	1.214
	—	—	B6*	—	(<i>b</i>)	<i>b</i>	—	<u>42.898</u>
	—	—	B6-Tla ^a *	—	(<i>a</i>)	<i>a</i>	—	1.117
	—	—	BALB/c*	—	(<i>c</i>)	<i>b</i>	—	<u>32.290</u>

IL-2 production of GT reactive hybridomas was measured as described in Table 1. EL4 and R1.1(T1a⁺) are mouse T-cell lymphoma lines derived from C57BL/6 (*H-2^b*) and C58 (*H-2^k*) strains, respectively. APCs were pulsed by 5 h coculture with GT and subsequent washing. A sample of each GT-pulsed APC line was fixed with glutaraldehyde.* Irradiated GT-pulsed splenocytes from strains B6 (*Tla^b*, *Qa-1^b*), B6-Tla^a (*Tla^a*, *Qa-1^a*) and BALB/c (*Tla^c*, *Qa-1^b*) were used at final concentration of 5×10^5 per well. ** Significant responses are underlined.*** *Tla* antigen is not expressed^{6,29}.

(kb), respectively) probably originated from the fusion partner BW5147 thymoma, as deduced from the Southern blot analysis. Clone DGT3 had the same two V-D-J β 2 rearrangements as its lymphoma ancestor BW5147 and an additional two in the germline configuration as judged by the position and intensity of the 5'-D β 2/J β 2-specific bands on the autoradiograph (data not shown). The C β 1 genes were either in germline or not completely rearranged, because both the C β and 5'-D β 1 probes detected two superimposable bands on Southern blots containing *Hind*III-digested DNA (data not shown). Thus, only the BW5147-specific V-D-J β -rearrangements could be found in the DGT3 clone. In the Southern blot, the C δ probe hybridized to DGT3 but not to BW5147 genomic DNA, detecting in addition to the 30 kb germ-line fragment, a smaller rearranged *Bam*HI DNA fragment (Fig. 2b). Because *Bam*HI sites flank the C δ gene, the double band suggests the presence of a C δ -containing pair of chromosomes in the DGT3 genome. This implies that only BW5147-specific α -gene rearrangements should be present in DGT3 cells (if they do not have more than four copies of chromosome 14), as α and δ TCR gene rearrangements seem to be mutually exclusive. The δ TCR chain transcripts in DGT3 appear as bands migrating at ~1.8 kb and ~1.5 kb, neither of which are detected in DGT1 cells (Fig. 2a). BW5147 carries three out-of-frame γ -gene rearrangements, only one of which seems to be transcribed (γ 1 gene; refs 9-11). In Southern blots with *Eco*RI-digested DNA, compound with the characteristic

pattern of BW5147, DGT3 showed an additional rearranged band of 5 kb with the C γ 1-probe (Fig. 2b). This suggests the existence of an additional γ -gene product in DGT3 cells, not normally found in BW5147 cells.

The DGT3 specificity (GT/Qa-1^b) could not be attributed to $\alpha\beta$ TCR, because the GT/A^d specific hybridoma DGT1 was not activated by GT in association with Qa-1^b, excluding the involvement of BW5147 $\alpha\beta$ TCR (Table 1, experiment 4). Also, DGT3 cells neither stained with pan- $\alpha\beta$ TCR reactive monoclonal antibody (H57.597; Fig. 1b; ref. 12) nor contained any other functional β -chain rearrangement other than the BW5147 type (not shown), excluding, in addition, any contribution by $\alpha\beta$ chains to the DGT3 TCR. The DGT3 clone expressed CD3 complex on the cell surface, presumably in association with $\gamma\delta$ TCR (Fig. 1b). The presence of the TCR heterodimer was demonstrated by staining DGT3 cells with pan- $\gamma\delta$ TCR reactive monoclonal antibody (3A10, Fig. 1; ref. 13) and SDS-PAGE of membrane-labelled DGT3 cell lysates, immunoprecipitated with antiserum specific for C γ 1/4. As shown in Fig. 2c, anti-C γ 1/4 antiserum, but not pan- $\alpha\beta$ TCR specific monoclonal antibody or anti-C γ 2 antiserum, immunoprecipitated from DGT3 cells a heterodimeric molecule with relative molecular masses ~34-36 and ~42-44 K corresponding to $\gamma\delta$ TCR (ref. 5). From the DGT1 clone, pan- $\alpha\beta$ TCR reactive monoclonal antibody precipitated a different heterodimeric molecule as compared with DGT3. Taken together, our results indicate that DGT3 cells

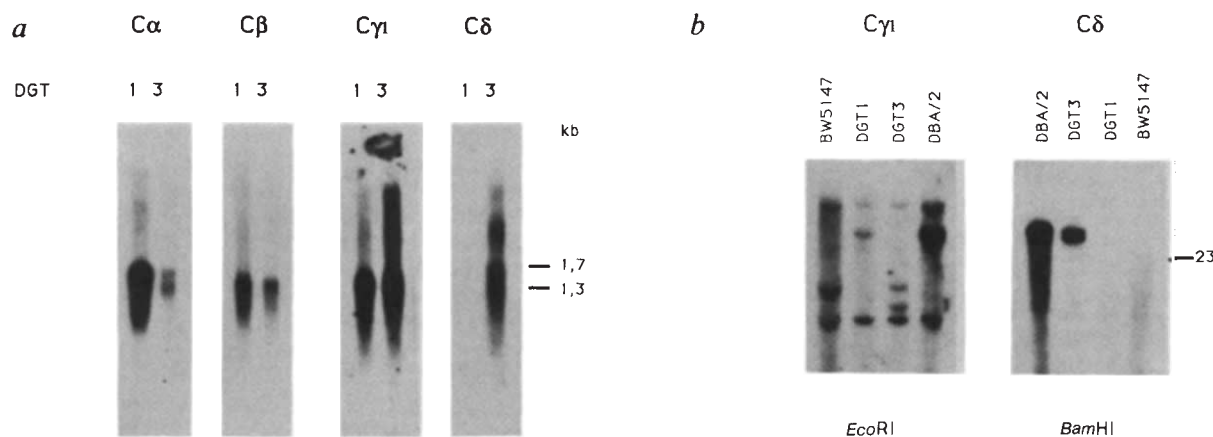
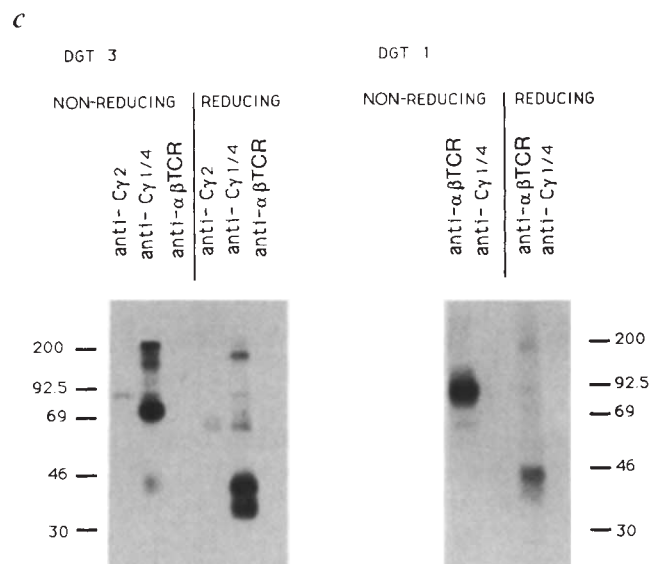


FIG. 2 *a*, Northern blot analysis of T-cell receptors in DGT1 and DGT3 clones. Poly (A)⁺RNA (~2 µg per lane) was separated on 1% formaldehyde gel, transferred to zeta probe nylon membrane (BioRad) and hybridized with the indicated probes. *b*, Southern blot analysis of the TCR-γ and δ genes in DGT1 and DGT3 clones. Gel electrophoresis of digested genomic DNA (10 µg per lane) and alkaline-transfer to zeta probe filters were done according to manufacturers' instructions (BioRad). *c*, SDS-PAGE analysis of immunoprecipitated TCRs from DGT1 and DGT3 clones. 3–5 × 10⁷ cells from each clone were surface-labelled by the lactoperoxidase method with ¹²⁵I and lysed with 0.5% NP-40. Proteins from the lysates were subsequently precipitated with pan-αβTCR reactive (HK57.597, ref. 12) monoclonal antibody or with antisera specific for Cγ-TCR: anti-Cγ1.4 or anti-Cγ2(Cγ1.2 and Cγ4 in ref. 5, respectively), and protein-A Sepharose 4B (Pharmacia) for 4 h. Immunoprecipitated proteins were analysed by 7–15% SDS-PAGE under reducing and non-reducing conditions as indicated. Relative molecular mass is indicated (×10³).

METHODS. RNA was isolated with a guanidinium isothiocyanate and CsCl gradient centrifugation procedure^{32,33}. Blots were hybridized in 0.5 M NaH₂PO₄, pH 7.2, 1 mM EDTA, 1% crystalline grade bovine serum albumin (Sigma) and 7% SDS (BRL), with 10–50 ng ml⁻¹ labelled probe (≥10⁸ c.p.m. µg⁻¹; random primer-labelling kit, Pharmacia), at 68 °C overnight. Membranes were washed once in 2 × SSC medium, 1% SDS at room temperature for 15 min and in 0.1 × SSC, 0.1% SDS once at room temperature, and once at 65 °C for 15 min. Exposure was 1–6 h with intensifying screens (Ilford Fast Tungstate). Sizes were estimated from the ethidium bromide-stained DriGest III marker (Pharmacia) run in parallel on the same gel. All probes were gel purified. Probe Cα is the 200 bp *EcoRV*-*Avall* fragment of the cDNA clone T1.2 (ref. 34); Cβ probe is the 300 bp insert of the β-chain clone 4.4 described previously³⁵; Cγ1 is the 1.25 kb *Kpn1*-



EcoRI fragment of the insert of the clone pTBDγ-4 isolated from the BDFL T-cell clone cDNA library (contains half of the Vγ1 entire Cγ and 3' untranslated region)⁹; Cδ is the 900 bp *EcoRI* insert of the δ-chain specific cDNA clone described previously³⁶. Immunoprecipitations and SDS-PAGE analysis were according to ref. 37.

express γδ TCR heterodimers on the cell surface, and these may play a role in the Qa-1 restricted recognition of GT.

This is the first report of Qa-associated recognition of foreign antigen by T cells. The question remains whether the recognition event has a physiological significance *in vivo*. The DGT3 specificity might originate from a minor population of T lymphocytes whose antigen recognition was Qa-1-dependent. Double-negative γδ TCR mature cytotoxic T-cell clones have broad, MHC-unrestricted specificity^{14–16}. Some of them seem to recognize allogeneic Tla and D class I molecules¹⁴. Although DGT3 had no crossreactivity to eight different common H-2 haplotypes (data not shown), it is still possible that this was not detected, because of an insufficiently large panel of potential stimulators. Conversely, the reported broad specificity of 'double negative' clones might represent crossreactivity of Qa-restricted T cells specific for unknown foreign antigen(s). It has recently been shown that the recognition of foreign antigens by human γδ T cells seemed to be restricted by monomorphic HLA determinants^{17–19}, but it remained unclear whether MHC restriction elements were encoded by the 'classical' or other ('non-classical') HLA genes²⁰. It might be possible, therefore, that in these cases foreign antigens were recognized in association with elusive human counterparts of mouse Qa-1 antigens. It is con-

ceivable that the possible physiological role of Qa-1-restricted recognition is not too distant from class I- or class II-restricted recognition by T cells. In contrast to previous findings, Qa-1 antigens were found to be widely expressed, in a similar way to classical class I molecules⁷. Thus, they might provide a context for the presentation of antigens for the 'first line of defence' T lymphocytes. Whether these cells bear only γδ TCRs remains to be answered. □

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Embryonic MAP2 lacks the cross-linking sidearm sequences and dendritic targeting signal of adult MAP2

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THE most prominent microtubule-associated protein of the neuronal cytoskeleton is MAP2. In the brain it exists as a pair of high-molecular weight proteins, MAP2a and MAP2b, and a smaller form, MAP2c, which is particularly abundant in the developing brain^{1–3}. High-molecular weight MAP2 is expressed in dendrites, where its messenger RNA is also located^{4,5}, but is not found in axons^{6–9}; it has been shown to be present in fine filaments that crosslink dendritic microtubules¹⁰. This correlates with the primary structure of high-molecular weight MAP2, which consists of a short carboxy-terminal tubulin-binding domain and a long amino-terminal arm¹¹, which forms a filamentous sidearm on reconstituted microtubules^{12–14}. Here we report that the high- and low-molecular weight forms of MAP2 are generated by alternative splicing and share the entire C-terminal tubulin-binding domain as well as a short N-terminal sequence. In contrast to high molecular weight MAP2, embryonic brain MAP2c lacks 1,342 amino acids from the filamentous sidearm domain. Furthermore, the mRNA for low molecular weight MAP2c is not present in dendrites, indicating that the dendritic targeting signal is specific for the high-molecular weight form.

We have previously described a MAP2 complementary DNA clone, 38a, which hybridizes to the 6-kilobase (kb) mRNA that encodes MAP2c (ref. 3). The nucleotide sequence of this clone was determined and the deduced amino-acid sequence was compared with that of high-molecular weight MAP2 from juvenile brain¹¹. The alignment of the deduced primary structures showed that MAP2c consists of a sequence from the

N-terminus of high molecular weight MAP2 joined to a sequence from the MAP2 C-terminus (Fig. 1). There is >95% amino-acid sequence similarity between rat MAP2c and the appropriate 5' and 3' portions of mouse MAP2. Allowing for inter-species variation, this suggests identity between the MAP2c sequence and their counterparts in high-molecular weight MAP2. The C-terminus of MAP2c thus contains all 3 of the 18-amino-acid repeated sequences that constitute the tubulin-binding domain of high-molecular weight MAP2¹¹.

To confirm that the sequence arrangement seen in clone 38a genuinely reflects the structure of MAP2c, we prepared cDNA probes from the 3', 5' and the joining regions of clone 38a, and used them to demonstrate their relationship to the 6-kb MAP2c and the 9-kb MAP2 mRNAs by northern blot analysis (Fig. 2). Figure 2a shows that both the 5' and 3' ends of clone 38a independently hybridized to the 6-kb MAP2c mRNA and also to the 9-kb MAP2 mRNA, confirming that MAP2c contains both N- and C-terminal sequences of the high-molecular weight MAP2. A northern blot of the same mRNA species with rat cDNA clone 19a (ref. 3), which encodes part of the sidearm domain of high-molecular weight MAP2, shows hybridization only to the 9-kb mRNA (Fig. 2a), confirming that this sequence is absent from MAP2c. In contrast, a synthetic oligonucleotide probe (31-mer) spanning the joining region 'J' of clone 38a hybridized specifically to the 6-kb MAP2c mRNA (Fig. 2b). As a control we subsequently hybridized the same northern blot with clone 19a without removing the 'J' probe, to reveal the 9-kb MAP2 mRNA (Fig. 2b). A 64-mer oligonucleotide probe derived from 5'-untranslated sequence of a rat high-molecular weight MAP2 clone also hybridized to both the 6-kb- and 9-kb mRNAs (data not shown), indicating that the sequence identity between high- and low-molecular weight MAP2 transcripts

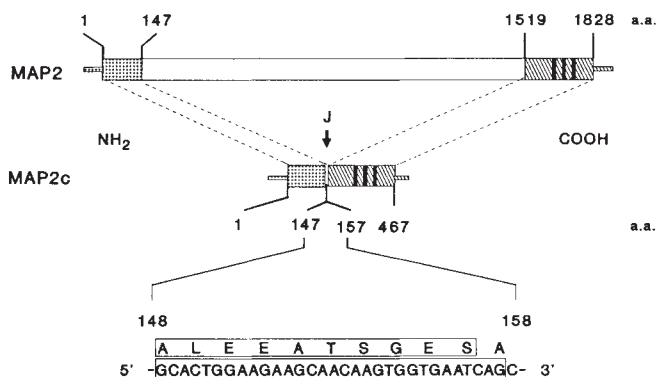


FIG. 1 Schematic representation of mouse high-molecular weight MAP2 and rat MAP2c protein sequences drawn to scale. Shaded boxes represent the N- and C-terminal amino-acid regions that are common to both proteins and are respectively 95% and 99% similar. The high sequence homology continues within the 3' and 5'-non-coding DNA-sequences adjacent to the open reading frames, showing 87% similarity at the 5' and 95% at the 3' end over 67 and 173 nucleotides, respectively. The three vertical black boxes indicate the repetitive sequences of the tubulin-binding domains¹⁰. The dotted lines indicate the alternative splicing of the primary transcript to give either MAP2 or MAP2c, with the result that 1,372 amino acids of the filamentous sidearm-domain (open box) are absent from MAP2c. The nucleotide sequence spanning the joining region J of MAP2c and the deduced amino-acid sequence are also shown. The sequence of the oligonucleotide used for northern blot hybridization (nucleotides 307–342) and the amino-acid sequence of the synthetic decapeptide used to raise antibodies are boxed. Single letter code: A, Ala; L, Leu; E, Glu; T, Thr; S, Ser; G, Gly. METHODS. The cDNA of the λ 38a (ref. 10) clone was subcloned into the EcoRI site of the Bluescript KS vector (Stratagene). The complete sequence was determined for both strands by dideoxy sequencing (Sequenase kit, United States Biochemical) using synthetic internal primers. DNA sequences were analysed using the software package from the University of Wisconsin Genetics Computer Group²⁴ and the FastP and FastA programs run on a Vax 8600.

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extends to the region upstream of the translation initiation codon.

As final confirmation that 38a is a MAP2c cDNA, and to demonstrate that the splicing region is present in the MAP2c protein, we raised an antiserum against a synthetic decapeptide (see Fig. 1) containing the joining-region sequence. Figure 2c shows western blots of juvenile rat brain supernatant, stained with either a monoclonal antibody against MAP2 that recognizes all the MAP2 forms (lane C) or the antibody against the decapeptide of the MAP2c joining region (lane J). Whereas the anti-MAP2 antibody strongly stained the high-molecular weight MAP2 form, the antibody against the MAP2c joining sequence selectively stained MAP2c.

Previous *in situ* hybridization experiments with a probe specific for the 9-kb MAP2 mRNA showed that this transcript is present in dendrites^{4,5}. We used the 31-mer oligonucleotide probe corresponding to the MAP2c joining region (J probe) to localize the 6-kb MAP2c mRNA *in situ*. Figure 3 shows *in situ* hybridization patterns with the J probe in the adult rat entorhinal cortex. Unlike the control C-sense oligonucleotide, the J probe hybridized in a punctate pattern characteristic of the distribution of cortical pyramidal neurons. There was no significant hybridization with the J probe in pyramidal cell primary dendrites, nor in layer I, where the high-molecular weight MAP2 mRNA is found⁵.

In both the developing (Fig. 4c) and the adult hippocampus (Fig. 4f), the J probe hybridized with neuronal cell bodies and not the dendrite-rich molecular layers of the hippocampal pyramidal cells and the granule cells of the dentate gyrus. In contrast, the 9-kb mRNA was found in these molecular layers

(Fig. 4a, e). Figure 4 also demonstrates the necessity of using the oligonucleotide probe to locate the MAP2c mRNA: the patterns of hybridization with 38a and 19a cDNA clones are indistinguishable (Fig. 4a, b).

Our results demonstrate that the low-molecular weight, embryonic brain form of MAP2 consists of the extreme N-terminal and C-terminal ends of the high-molecular weight MAP2 coding sequence. A large portion of the central region of high-molecular weight MAP2, constituting most of the sidearm domain that projects from and may crosslink microtubules, is missing. The primary structure of MAP2c, together with the evidence that both the 9-kb and 6-kb MAP2 mRNAs are derived from a single MAP2 gene^{3,17}, indicate that high- and low-molecular weight MAP2s are the result of alternative splicing of a primary MAP2 gene transcript. The embryonic MAP2c results from the splicing out of a sequence coding for 1,372 amino acids of the central domain of mature MAP2. This alternative splicing is developmentally regulated, for MAP2c and its 6-kb mRNA are more abundant during brain development than in the adult brain^{1,3}. In the rat¹, quail¹⁸ and *Xenopus*¹⁹, the disappearance of MAP2c occurs abruptly, and coincides with the stabilization of neuronal morphology.

The expression of the various MAP2 forms is temporally as well as spatially regulated. High-molecular weight MAP2 is present in dendrites⁶⁻⁹, where its 9-kb mRNA is also localized^{4,5}, in contrast to MAP2c, which is found in axons^{18,19}. This suggests that high-molecular weight MAP2 contains unique sequences, absent from MAP2c, that are responsible for its specific expression in dendrites and not in axons. The presence of the 9-kb high-molecular weight MAP2 mRNA in dendrites further

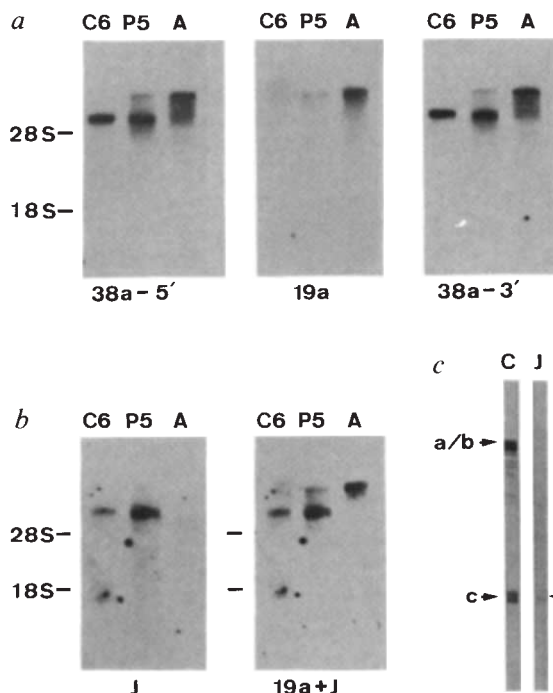


FIG. 2 Verification of the MAP2c and high-molecular weight MAP2 transcript structure by northern blot analysis. Northern blot hybridization of poly(A)⁺ mRNA from C6 glioma cells (C6), postnatal day-5 (P5) baby rats and adult rats (A). a, DNA-sequences from the 3'-end (left) and the 5'-end (right) of MAP2c, and from the (high molecular weight) MAP2-specific region encoding the filamentous sidearm (clone 19a (ref. 3), middle) were used to probe the blots. b, Northern blot hybridization (left) with the synthetic oligonucleotide from the joining region J of MAP2c (Fig. 1c). The same blot was subsequently hybridized in addition with the high-molecular weight MAP2-specific clone 19a to confirm the presence of the 9-kb mRNA that does not hybridize with the J probe (right). The positions of 18S and 28S ribosomal RNAs are indicated. c, Immunostaining of heat-stable MAPs from P6 (postnatal day 6) rat brain-supernatant with the antiserum against the MAP2c J peptide. The blot on the left was stained with the C monoclonal antibody that recognizes both high- and low-molecular weight MAP2 (arrows). The blot on the right shows the staining with the mouse polyclonal serum raised against the joining peptide J (see Fig. 1c), which reacts selectively with MAP2c (arrow).

METHODS. For northern blot analysis, poly(A)⁺ RNA was denatured by glyoxal at 50 °C, electrophoresed on 1% agarose gels and transferred onto Hybond-N (Amersham) nylon membranes. The RNA was fixed by UV irradiation. Pre-hybridization was at 42 °C in 50% formamide, 5 × SSC, 50 mM sodium phosphate, pH 6.8, 0.5% SDS, 10% dextran sulphate and 200 μg ml⁻¹ heat denatured heterologous DNA. Hybridization was carried out in the same solution containing 2 × 10⁶ c.p.m. ml⁻¹ of heat-denatured probe. The cDNA fragments were labelled by random oligonucleotide priming. Filters were washed twice for 20 min in 2 × SSC, 0.1% SDS at 60 °C, and for 20 min in 0.2 × SSC, 0.1% SDS at 60 °C. The probes used for hybridization were: a 230-basepair (bp) *EcoRI*-*PstI* fragment from the 3'-end of clone 38a, spanning amino acids 170-466; a 1,064-bp *TthI*-*EcoRI* fragment from the 5'-end encoding amino acids 45-121; and the 1,313-bp *EcoRI* insert of clone 19a. Hybridization with [³²P]dATP (2 × 10⁶ c.p.m. ml⁻¹)-kinased oligonucleotide (31-mer) was performed at 40 °C in 6 × NET, 5 × Denhardt's solution, 0.5% SDS and 200 μg ml⁻¹ transfer RNA. Filters were washed twice, for 20 min with 2 × SSC and 0.1% SDS at 50 °C and for 15 min with 0.2 × SSC and 0.1% SDS at 37 °C. The decapeptide of the MAP2c joining region (Ala-Leu-Glu-Glu-Ala-Thr-Ser-Gly-Glu-Ser) was synthesized by the Fmoc-polyamide method²⁵ on a CRB-pepsynthesizer. The peptide was coupled to keyhole-limpet haemocyanin before immunization. Production of antiserum in mouse and immunoblotting were performed as described³. The serum was diluted 1:100 and the supernatant of the antibody against MAP2 that recognizes all the MAP2 forms diluted 1:10.

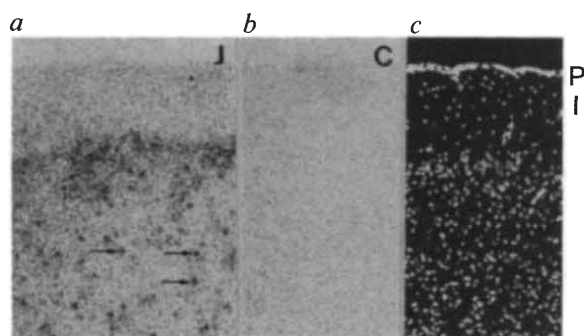


FIG. 3 *In situ* hybridization of the oligonucleotide spanning the MAP2c joining region to coronal sections through the entorhinal cortex of an adult rat brain. *a*, The bright field image shows the distinctive punctate hybridization pattern in the cell bodies of neurons in the cortex obtained with the 31-mer J oligonucleotide. Layer I (I) and the pia (P) show no significant hybridization (see *c*). *b*, Hybridization of an adjacent section with a control 27-mer sense C-oligonucleotide gave only uniform background labelling. *c*, Location of cells bodies shown by staining with Hoechst nuclear dye.

METHODS. *In situ* hybridization using the MAP2 (19a) and MAP2c (38a) cDNA inserts was as described previously^{4,5}. For hybridization with the 31-mer MAP2c-J oligonucleotide and the 27-mer control sense-probe the sections were thawed and prehybridized for 1 h at 37 °C (in 25–40 μ l per slide) in 50% formamide, 4 $\times$ SSC, 20 mM sodium phosphate, pH 6.8, 1 $\times$ Denhardt's solution, 0.2% SDS, 10% dextran sulphate, 20 mM β -mercaptoethanol and 100 μ g ml⁻¹ each of heat-denatured single-strand DNA, tRNA and poly(A) DNA. The hybridization was carried out overnight in the same solution containing 1 $\times 10^6$ c.p.m. per 100 μ l of [α -³⁵S]dATP-labelled probes. The sections were then washed three times for 20 min in 1 $\times$ SSC at room temperature, three times for 20 min in 1 $\times$ SSC at 37 °C and twice for 20 min in 0.5 $\times$ SSC at room temperature, and dehydrated. Following exposure to β -max (Amersham) film for 1–3 days, the slides were dipped in K5 nuclear emulsion (Ilford) and exposed for 7–10 days. The sections were further processed as described previously⁸.

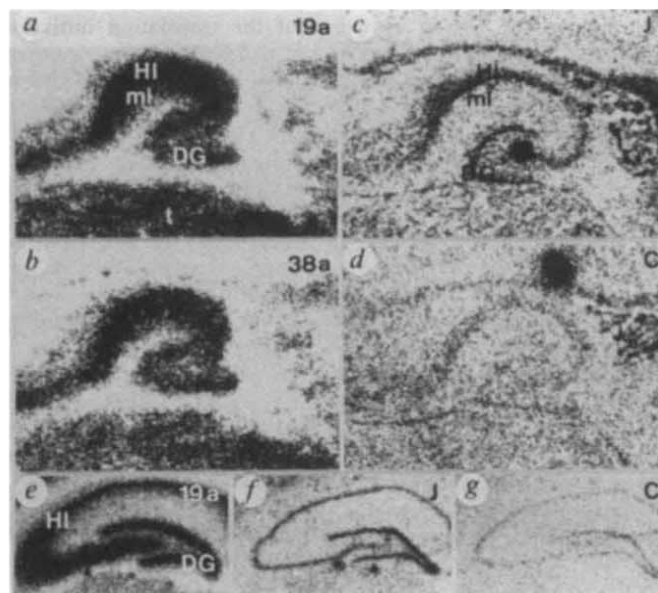


FIG. 4 Localization of the 9-kb and 6-kb mRNAs of MAP2 in the developing (*a-d*) and adult (*e-g*) rat hippocampus. The hybridization patterns in the developing hippocampus are indistinguishable with the two cDNA probes 19a (*a*) and 38a (*b*); both show diffuse hybridization in the molecular layer (ml) of the hippocampal pyramidal cells (HI) and in the dentate gyrus (DG). The 6-kb-specific J oligonucleotide probe (*c*) hybridizes only within the cell-body layers of the hippocampus and dentate gyrus. Sections hybridized with a control oligonucleotide C (*d*) show a background staining mainly within the molecular layer. A similar segregation of high- and low-molecular weight MAP2 mRNAs is seen in the adult. The 19a cDNA (*e*) hybridizes with a diffuse pattern in both the hippocampus (HI) and dentate gyrus (DG), in contrast to the 6-kb-specific J oligonucleotide (*f*), which hybridizes specifically to the neuronal cell bodies. The sense oligonucleotide C (*g*) was used as a control.

suggests that the dendritic targeting signal may be present in the mRNA itself. These sequences could be related to the long central portion of high-molecular weight MAP2 that is missing from MAP2c, or alternatively could reside in 5' or 3' non-coding regions of the MAP2 transcript. Examples of controlled cytoplasmic distribution of mRNAs based on 3'-untranslated sequences have recently been described for maternal transcripts in *Xenopus* oocytes²⁰ and *Drosophila* embryos²¹.

We have recently found that MAP2c is persistently expressed in areas of the adult brain where neuronal growth continues. Levels of MAP2c remain high in retinal photoreceptor cells²² that regenerate their outer segments, and in the olfactory bulb, where neurite outgrowth and synaptogenesis persist in the adult²³. Thus the expression of MAP2c seems to be important for neuronal morphogenesis. Because MAP2c is essentially high-molecular weight MAP2 with 1,372 amino acids missing from the middle, its selective expression during neuronal differentiation is probably related to the function of this central portion. The significance of this enormous deletion may be related to the demonstrated involvement of the high-molecular weight MAP2 'sidearm' domain in crosslinks between microtubules¹⁰. Because the isolated C-terminal repeats of MAP2 have been shown to bind to tubulin polymers¹¹, MAP2c, which contains the entire MAP2 tubulin-binding domain, should bind to microtubules with the same efficiency as high-molecular weight MAP2. In the developing neuron, MAP2c would thus occupy MAP2 binding sites on microtubules without providing the extended sidearm domain. One possible consequence of this is that the expression of MAP2c reduces the degree of cytoskeletal crosslinkage during axonal and dendritic growth, thus

providing a more plastic structural state during neuronal morphogenesis. The maturation of neuronal shape would then involve the disappearance of MAP2c and the appearance of an additional high-molecular weight MAP2, MAP2a^{1,15,16}, to provide additional crosslinks in the neuronal cytoskeleton and hence stabilize the adult form of the dendrites. □

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Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity

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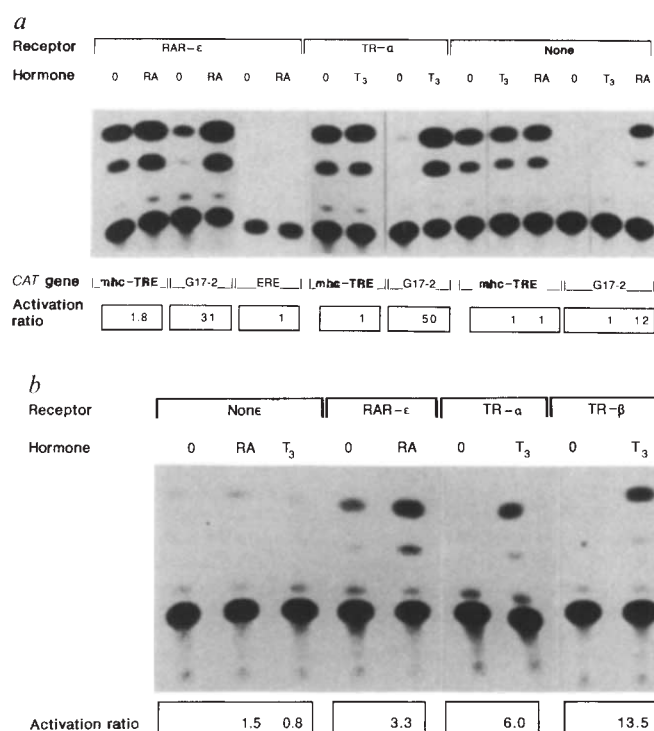
BOTH thyroid hormone (T_3) and retinoic acid signal essential steps in development, differentiation and morphogenesis. Specific nuclear receptors for these ligands have recently been cloned¹⁻⁸. Previously we have noted a close homology between the DNA-binding domains of the ϵ -retinoic acid receptor (RAR- ϵ , also designated RAR- β), the thyroid hormone receptors and the oestrogen receptor^{2,3}. We have now found that RAR- ϵ is very efficient at inducing transcription from two distinct thyroid-hormone responsive elements (TREs). Transcription induced by ligand-activated RAR- ϵ from a TRE can, however, be repressed by thyroid-hormone receptor in the absence of its ligand. Conversely, in the presence of its ligand, thyroid-hormone receptor will activate transcription from a TRE irrespective of the presence of unbound RAR. The use of hybrid receptors has shown that the DNA-binding domain of RAR is the essential target for inhibition by thyroid-hormone receptors. These data, together with *in vitro* DNA-binding studies, suggest that thyroid-hormone receptors may have dual regulatory roles: in the presence of hormone they function as TRE-specific transcriptional activators; in the absence of hormone, however, they can function as TRE-specific repressors.

FIG. 1 RAR- ϵ and the endogenous F9 cell RAR activate transcription from thyroid-hormone responsive elements. *a*, F9 cells were co-transfected² with 5 μ g expression vectors PECE-RAR- ϵ or PECE-TR- α and 20 μ g reporter plasmids G17-2-CAT or MHC-CAT. As a control, cells were also transfected with the reporter plasmids only. The G17-2-CAT construct contains three copies of a synthetic TRE derived from the rat growth-hormone gene¹⁹. The *mhc* construct contains nucleotides -163 to -81 upstream of the CAP site of the rat α -*mhc* gene¹⁰. Activation ratio is the ratio of relative CAT activity after hormone induction to relative CAT activity without hormone induction. The mean of at least three experiments is given. *b*, RAR- ϵ activates transcription from a TRE in CV-1 cells. Cells were co-transfected with PECE-RAR- ϵ , PECE-TR- α or PECE-TR- β (5 μ g each) as indicated, and G17-2-CAT construct (20 μ g). Final concentrations of hormone were: retinoic acid (RA), 6×10^{-7} M; and T_3 , 10^{-7} M. Mean activation for three separate experiments is given.

METHODS. F9 cells (2×10^6) were cultured as a monolayer on gelatine-coated dishes in alpha-ME medium (GIBCO), supplemented by 10% FCS, glutamine and nucleosides as described²⁰. Cells were fed the medium containing charcoal-treated FCS, 2-5 h before transfection; 24 h after transfection, cells were fed with charcoal-treated medium together with the appropriate amount of hormone (final hormone concentrations, 5×10^{-8} M); 48 h after transfection, cells were collected, lysed and assayed as described²¹. CV-1 cells were plated at 1.8×10^5 per dish 12-16 h before transfection in DMEM medium (GIBCO) and 10% fetal calf serum. For accurate determinations of chloramphenicol acetylation, the separated reaction products were cut out from silica plates and quantified in a scintillation counter. Plasmid constructions: PECE-RAR- ϵ was obtained by cloning a *Bam*HI-*Eco*R1 fragment (containing the complete coding region of RAR- ϵ) from clone BI-RAR (ref. 2) (BI designates BlueScript; Stratagene) into the *Bgl*II and *Eco*R1 sites of the PECE expression vector²². To obtain PECE-TR- α , a 2-kilobase (kb) *Eco*R1 fragment of the TR- α clone prbeA12⁵ was cloned into the *Eco*R1 site of PECE (ref. 22). For construction of PECE-TR- β , a 1.6-kb *Eco*R1 fragment of the human *c-erb-A*- β clone⁴ was ligated into the *Eco*R1 site of the PECE vector. G17-2-CAT was derived from G17-2 Luc (C. K. Glass *et al.*, manuscript in preparation) by exchange of the reporter gene.

We investigated whether TREs and/or the oestrogen-responsive element (ERE) could be activated by RAR- ϵ . The transcriptional activation of reporter genes of these elements was monitored using constructs in transient transfection assays. The constructs contained the chloramphenicol acetyltransferase reporter gene (CAT) linked to the TRE of the myosin heavy chain gene (*mhc*)¹⁰, the synthetic palindrome TRE (G17-2)¹¹ or the ERE of the vitellogenin gene¹². Co-transfection of F9 teratocarcinoma cells with a RAR- ϵ expression vector and either of the two TRE-CAT constructs resulted in the induction of CAT activity that was dependent on the presence of retinoic acid. By contrast, retinoic acid did not significantly stimulate CAT activity when the RAR- ϵ expression vector was co-transfected with the ERE-*tk*-CAT construct (Fig. 1*a*). In the presence of retinoic acid, both the TRE constructs yielded very similar levels of CAT activity. In the absence of retinoic acid, however, CAT activity was constitutively stimulated at a high level by the *mhc*-TRE-CAT construct, but at a low basal level by the G17-2-CAT construct. The G17-2 construct, therefore, behaved as an ideal retinoic-acid inducible gene, in that an increase in CAT activity induced by retinoic acid of more than 30-fold was observed (Fig. 1). When the thyroid hormone α -receptor (TR- α) was co-transfected with the reporter constructs in the presence of T_3 , similar levels of CAT activity were obtained (Fig. 1*a*).

F9 cells have been established as a cellular model for retinoic-acid dependent differentiation¹³. We therefore investigated whether the endogenous F9 RAR could also activate the TRE-CAT genes by transfecting F9 cells with the reporter genes alone. A 12-fold increase in CAT activity was induced in the presence of retinoic acid on transfection with the G17-2 construct. This was considerably lower than the increase observed when F9 cells were co-transfected with RAR- ϵ and the G17-2 construct, indicating that the endogenous RAR is limiting in concentration. The high basal level of CAT activity observed with the *mhc*-TRE construct prevented observation of a clear induction effect by



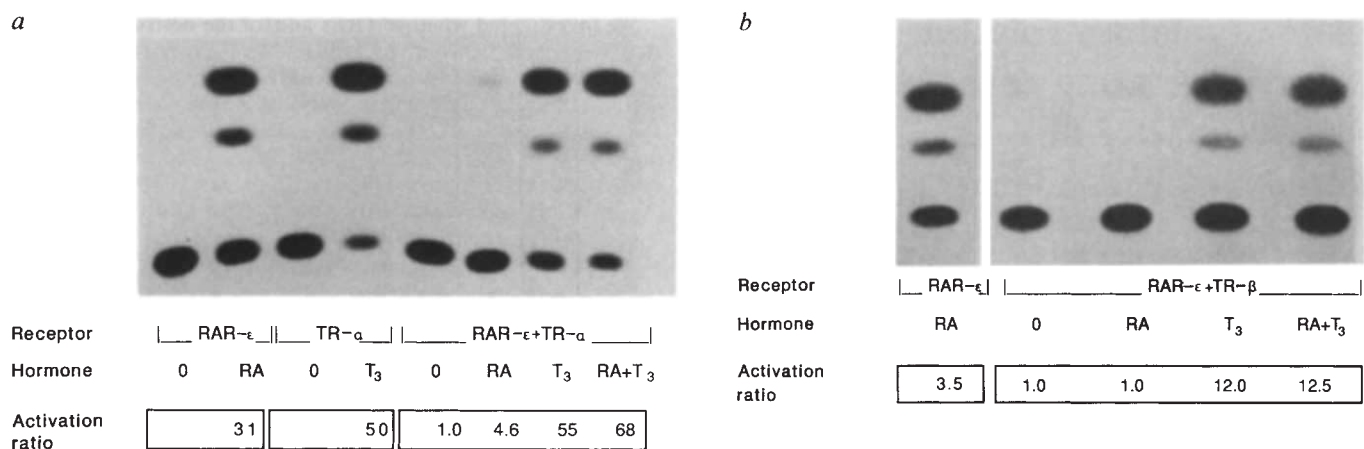


FIG. 2 TRs repress the activity of RAR-ε in F9 and CV-1 cells. **a**, PECE-RAR-ε (5 μg) and PECE-TR-α (5 μg) were co-transfected together with G17-2-CAT (20 μg) into F9 cells. As a control, each receptor was also co-transfected with G17-2-CAT individually. Hormones were added as indicated. **b**, PECE-TR-

β (2.5 μg) and PECE-RAR-ε (2.5 μg) were co-transfected with G17-2-CAT (20 μg) into CV-1 cells. As a control, PECE-RAR-ε was also co-transfected with G17-2 individually. Hormones were added as indicated. CAT activity was assayed and quantified as described in Fig. 1.

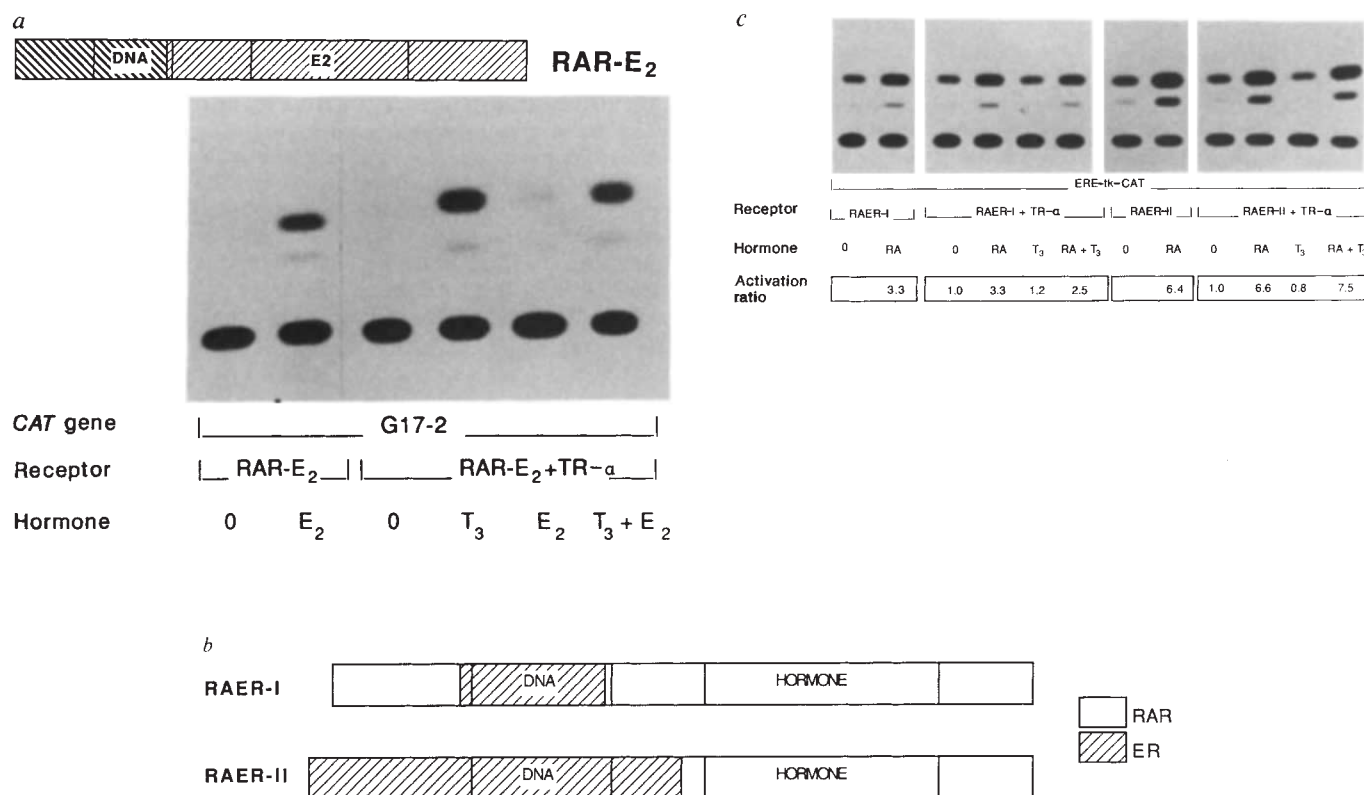


FIG. 3 Defining RAR regions sensitive to TR repression by hybrid-receptor analysis. **a**, The hybrid receptor RAR-E₂ is shown at the top. RAR-E₂ (5 μg) and TR-α (5 μg) were co-transfected with G17-2-CAT (20 μg) into F9 cells. As a control, RAR-E₂ (5 μg) and G17-2 (20 μg) were also co-transfected. Hormones were added as indicated at concentrations as above. Oestradiol (E₂) was added to a final concentration of 5×10^{-8} M. **b**, The hybrid receptor RAER-I contained the oestrogen receptor (ER) DNA-binding domain (DNA in figure) as described previously². RAER-II contains amino acids 1–287 of the human oestrogen receptor, which includes the DNA-binding domain up to the hinge region, and amino acids 169–448 of RAR_ε, which includes a portion of the hinge region and the hormone-binding domain. Details of the construction to be published elsewhere (M.T. and M.P., manuscript in preparation). **c**, RAER-I and RAER-II activate transcription from an ERE-*tk*-CAT reporter gene in the presence of TR-α. CV-1 cells were co-transfected with PECE-

RAER-I or PECE-RAER-II (2.5 μg) and PECE-TR-α (2.5 μg), together with ERE-*tk*-CAT (20 μg) reporter gene as above. The ERE-*tk*-CAT contained an ERE¹². As a control, transfection with RAER-I and RAER-II are also shown. Transfected cells were treated with retinoic acid (RA) (6×10^{-7} M), T₃ (10^{-7} M), or retinoic acid and T₃ as indicated.

METHODS. Construction of RAR-E₂. BI-RAR (ref. 2) was digested with *Apal* and *HincII*. This removes a 1-kilobase (kb) fragment with the hormone-binding domain and the terminus of the RAR-ε. A 1.15-kb *NaeI*-*Apal* fragment of the human ER clone BI-ER (ref. 1) was ligated to the RAR-ε fragment that encodes the N-terminal and DNA-binding domains. DNA sequencing confirmed the in-frame connection between the RAR DNA-binding domain and the oestrogen receptor hinge region. For expression, a 1.8-kb *Bam*HI-*SalI* fragment from BI-RAR-E₂ was cloned into the *Bgl*II and *Sal*I sites of PECE.

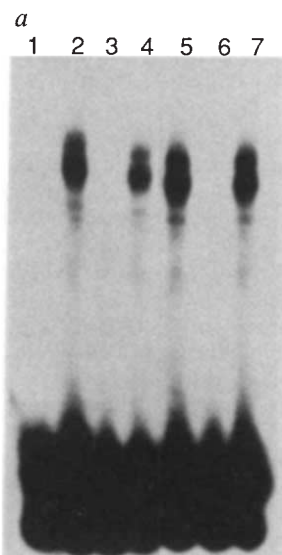
the endogenous RAR. No induction of CAT was seen in the presence of T_3 , suggesting that F9 cells do not contain TRs. To investigate the retinoic-acid concentration range necessary for RAR-dependent TRE activation, we monitored CAT activity of the G17-2 construct at retinoic-acid concentrations 3×10^{-11} M– 3×10^{-6} M. We observed that CAT activity, which is dependent on TREs, can be induced by RAR- ϵ , or F9 RAR, at retinoic-acid concentrations in the range of 0.1–1.0 nM (data not shown). These concentrations are well within the physiological range at which retinoids function¹³. To ensure that RAR-dependent TRE activation was not unique to one cell line, we repeated the same experiments using CV-1 cells. Similar results to those for the F9 cells were obtained (Fig. 1b).

Our data are therefore consistent with the assumption that TREs are highly efficient retinoic-acid responsive elements, suggesting that retinoic acid and T_3 modulate gene transcription, through their respective nuclear receptors, from a common family of responsive elements. This implies that certain genes in cells containing both receptors should be inducible by either T_3 or retinoic acid. To test this hypothesis and to investigate possible positive and/or negative interactions of TR and RAR, both receptors were co-transfected into the same cells, together with a TRE-CAT construct. High CAT activity was observed in the presence of both ligands (T_3 and RA). Surprisingly, no activity (or very low activity) was observed when only RA was added. These results were obtained in both F9 (Fig. 2a) and CV-1 cells (Fig. 2b), and indicate that in the presence of both receptors, TR alone controls the regulation of transcription from TREs. In the absence of T_3 , TRs prevent the activation of TREs by RARs, whereas in the presence of T_3 , TRs activate TREs

and also allow some degree of positive cooperativity with RARs. This dominating regulatory effect can be carried out by both the two closely related thyroid-hormone receptors TR- α and TR- β , on RAR- ϵ (Fig. 2) and the F9 endogenous RAR. Significant repression of RAR- ϵ activity in F9 cells was observed with RAR- ϵ to TR- α ratios of between 1:1 and 4:1. Whole-cell binding assays showed, however, that RAR- ϵ expression is not reduced by the presence of TRs (data not shown).

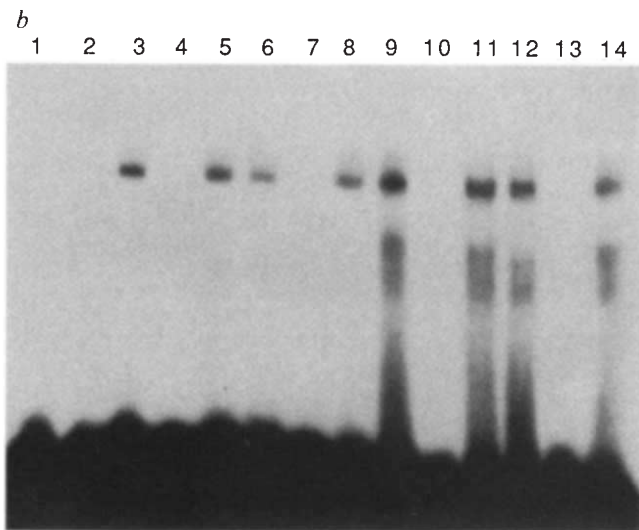
To define the region(s) of RAR sensitive to repression by TRs, hybrid receptors were constructed. The hybrid RAR-E₂ receptor shown in Fig. 3a contains the RAR- ϵ DNA-binding domain and the oestrogen receptor hormone-binding domain. This receptor was able to activate a TRE-CAT gene in the presence of oestradiol (Fig. 3a). When co-transfected with TR- α (or TR- β), the activity of RAR-E₂ was inhibited by TRs almost as efficiently as was RAR- ϵ , despite the altered hormone specificity (Fig. 3a). The activity of the hybrid receptors RAER-I and RAER-II (Fig. 3b), which contain oestrogen receptor DNA-binding domains and RAR- ϵ hormone-binding domains, was not inhibited by TRs (Fig. 3c). In addition, glucocorticoid receptors did not inhibit RAR activity (data not shown). Our data, therefore, suggest that the repression of RAR activity by non-activated TRs is mediated by specific interference with the binding of RAR to DNA. Although the mechanistic details of TR repression still need to be elucidated, in the simplest model the TRs would have a dual regulatory role: in the absence of hormone they bind to TREs and function as TRE-specific repressors, preventing other related receptors from activating the TREs; in the presence of hormone they act as TRE-specific transcriptional activators.

FIG. 4 DNA binding of TRs and RAR- ϵ *in vitro*. *a*, Thyroid-hormone receptor binds to TRE in the absence and presence of T_3 . Nuclear extracts from GC cells, known to contain TR (ref. 11), were incubated with 32 P-labelled oligonucleotide encoding G17-2. Gel retardation analysis was used to investigate specific binding. Lane 1, migration of 32 P-labelled TRE; lanes 2–4, GC-cell extract in the absence of T_3 ; lanes 5–7, extract in the presence of T_3 (5×10^{-8} M). All extracts were incubated with 32 P-labelled TRE; in lanes 3 and 6, a 50-fold excess of unlabelled TRE was added; in lanes 4 and 7, a 50-fold excess of a non-specific 30-



base pair (bp) oligonucleotide was added. *b*, *In vitro* synthesized RAR- ϵ and TR- β bind TRE in the presence and absence of ligand. Lane 1, 32 P-labelled TRE; lane 2, TRE plus reticulocyte lysate; lanes 3–5, *in vitro* synthesized RAR- ϵ in the absence of RA; lanes 6–8, RAR- ϵ plus retinoic acid. Lanes 9–11, *in vitro* synthesized TR- β in the presence of T_3 ; lanes 12–14, TR- β in the presence of T_3 . In lanes 4, 7, 10 and 13, a 50-fold excess of cold TRE was added to the incubation mixture; in lanes 5, 8, 11 and 14 a 50-fold excess of non-specific 30-bp oligonucleotide was added.

METHODS. GC cells were grown in DMEM medium with 5% charcoal-treated fetal bovine serum and 10% charcoal-treated horse serum in the absence or presence of 10 nM T_3 . Cell extracts were prepared in a buffer containing 20 mM HEPES, pH 7.9, 0.4 M KCl, 2 mM DTT and 20% glycerol. Aliquots with 2 μ g total protein were incubated with 32 P-labelled G17-2 and 200 μ g ml⁻¹



poly(dI-dC) in a reaction mixture containing 10 mM HEPES, pH 7.9, 80 mM KCl, 1 mM DTT, 2.5 mM MgCl₂ and 10% glycerol. The reaction mixture was incubated at 25 °C for 20 min, then loaded on a 5% non-denaturing polyacrylamide gel containing 10 mM HEPES, pH 7.9. Electrophoresis was at 4 °C, 120 V (6.5 V cm⁻¹) for 4 h with continuous circulation of the buffer (25 mM HEPES, pH 7.9). Synthetic oligonucleotide G17-2 was radioactively labelled using the Klenow fragment of DNA polymerase I. After purification by 10% polyacrylamide gel, ~3 ng of labelled G17-2 was used for each binding assay. For competition experiments, 150 ng of cold competitor was added before addition of 32 P-labelled G17-2. Translated products of TR- β and RAR- ϵ in the presence or absence of its ligand were prepared *in vitro* as described². Each binding assay used 50 mM KCl and 1 μ g poly(dI-dC) per 5 μ l *in vitro* synthesized protein.

We performed gel retardation experiments to determine whether TRs can bind to TREs in the absence of hormone. In one set of experiments nuclear extracts were prepared from GC cells that were known to contain TRs¹¹. Extracts from cells grown in either the presence or absence of T₃ were all found to retain the labelled TREs. The specific retardation could be displaced by unlabelled TREs but not by non-specific DNA. Similar results were obtained when TR- β was synthesized by *in vitro* transcription-translation¹. A specific protein-DNA complex was observed which was independent of the presence or absence of the hormone (Fig. 4b, lanes 9-14). Similar results were obtained with TR- α (data not shown). Interestingly, RAR- ϵ also specifically retarded the TREs in the presence or absence of hormone (Fig. 4b, lanes 3-8).

The results of this study demonstrate that both the human RAR- ϵ and the F9 endogenous RAR can mediate signal-dependent induction of transcription from TRE-containing promoters. These data are consistent with the observation that RAR- α can activate TREs from the rat growth-hormone gene¹⁴. Our findings, that two different types of receptors (TRs and RARs) act through the same DNA sequence, are also reminiscent of earlier discoveries that progesterone, glucocorticoid and mineralocorticoid receptors all recognize identical response elements. Pharmacologically, T₃ as well as retinoic acid are both effective in the treatment of acne¹⁵.

The regulatory mechanism we describe here in which TRs can act as TRE-specific repressors in the absence of ligand is novel and may also be an important feature of other nuclear receptors that have been shown to bind their specific response elements in the absence of hormone^{16,17}. This repression mechanism seems to be essential for the thyroid-hormone response, as isoforms of TRs that have been isolated (ref. 18, and T. Hermann, G.G., X.Z. and M.P., manuscript in preparation) seem to act as TRE-specific repressors only. A mechanism by which nuclear receptors can act as both repressors and activators specific for a particular response element would greatly increase the specificity of gene regulation. This mechanism could provide a new level of regulation by negative control for all responsive elements with overlapping receptor specificity. □

Note added in proof. While this paper was being reviewed, Damm *et al.*²³ published a similar observation on the dual regulatory role of TR- α and also showed that the *v-erb-A* protein, which is known not to bind T₃ (ref. 3), is a TRE-specific repressor.

Similarities between prokaryotic and eukaryotic cyclic AMP-responsive promoter elements

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ORGANISMS as diverse as bacteria and man contain genes that show transcriptional induction when the intracellular concentration of cAMP is increased^{1,2}. This regulated transcriptional response is mediated through specific promoter elements located, in general, upstream from the transcription start site. In *Escherichia coli* the element responsible for cAMP-mediated transcriptional induction is the binding site for the cAMP-receptor protein (CAP). In mammalian cells the cAMP regulatory element is composed of one or more binding sites for various transcription factors². In many instances the cAMP regulatory element contains binding sites for a family of proteins referred to as ATF. Here we provide evidence that some prokaryotic and mammalian cAMP-response elements are functionally related. First, we show that mammalian ATF binds specifically to some *E. coli* CAP sites, and conversely *E. coli* CAP binds specifically to some mammalian ATF sites. Second, we demonstrate that an *E. coli* CAP binding site can confer cAMP-inducibility onto a mammalian gene when assayed in transfected mammalian cells.

Both bacteria and mammalian cells contain genes that are transcriptionally activated by cAMP. The mechanism of this regulated transcription response is, however, quite different in the two systems. In bacteria, cAMP-inducible transcription is mediated through an activator protein referred to as the cAMP receptor protein (CAP or CRP). cAMP binds to CAP, converting it from an inactive form to one that can bind DNA and activate transcription¹.

In mammalian cells cAMP serves as a second messenger that transmits extracellular signals through a pathway involving the cAMP-dependent protein kinase². The cAMP-responsive element in mammalian gene promoters is referred to as a cAMP regulatory element (CRE). The CRE is necessary and in some instances sufficient for cAMP-inducibility. Many mammalian CREs are composed of one or more sites for a transcription factor referred to as either ATF (refs 3, 4) or CREB (ref. 5). Recent studies have shown that ATF is not a single factor but rather a family of related proteins⁶. Therefore, the term ATF is used here to indicate a DNA-binding activity and does not imply a specific protein.

ATF is an apparently ubiquitous transcription factor: proteins with ATF DNA-binding specificity are widely distributed throughout eukaryotes⁷. An ATF consensus binding site, 5'-GTGACGT_{CG}^{AA}-3', has been derived in which the 5'-TGACG-3' motif (ATF core) is the most highly conserved portion (see ref. 4). In addition, the specific bases flanking ATF sites⁸, and the context of ATF sites within a promoter^{9,10} can strongly influence the transcriptional response.

The *E. coli* CAP binding site is complex, but all CAP sites contain a highly conserved portion 5'-TGTTGA-3' (reviewed in refs 1, 11). In examining a collection of *E. coli* CAP binding sites^{1,11}, we and others^{2,12,13} recognized that in a number of instances they contained perfect or almost perfect ATF sites (Fig. 1). Also, alignment of the ATF consensus and *E. coli* CAP sites reveals that the essential guanosine contacts of the two proteins are also highly related (Fig. 1). We therefore sought to determine whether mammalian ATF sites and *E. coli* CAP sites might also be functionally related.

First, we asked whether purified mammalian ATF could in fact bind to the putative ATF sites present within some CAP sites. ³²P-labelled DNA probes containing the CAP sites were

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both prokaryotes and eukaryotes can work when positioned at a large distance from the transcription start site (reviewed in ref. 15). *E. coli* RNA polymerase and eukaryotic RNA polymerase II are both multisubunit enzymes, and the amino-acid sequence of some of these subunits has been conserved between these organisms¹⁶. Finally, both prokaryotes and eukaryotes have activator proteins that function after binding to specific promoter elements. Also, there are structural similarities between some prokaryotic and eukaryotic activators both in the DNA binding domains, for example, helix-turn-helix¹⁷; and in the activating regions, for example, negative charge/phosphorylation^{18,19} (reviewed in ref. 15).

We have shown that at least some eukaryotic ATF sites are functionally related to some prokaryotic CAP sites. Nevertheless, these elements are not identical and we do not wish to imply that all ATF sites are CAP sites, or that all CAP sites are ATF sites. It is difficult to predict how many CAP sites are also ATF binding sites, as saturating mutagenesis of an ATF binding site has not yet been performed. It seems more than coincidental, however, that there are both sequence and function similarities between promoter elements that mediate analogous transcriptional responses. Our results raise the possibility that the cAMP-

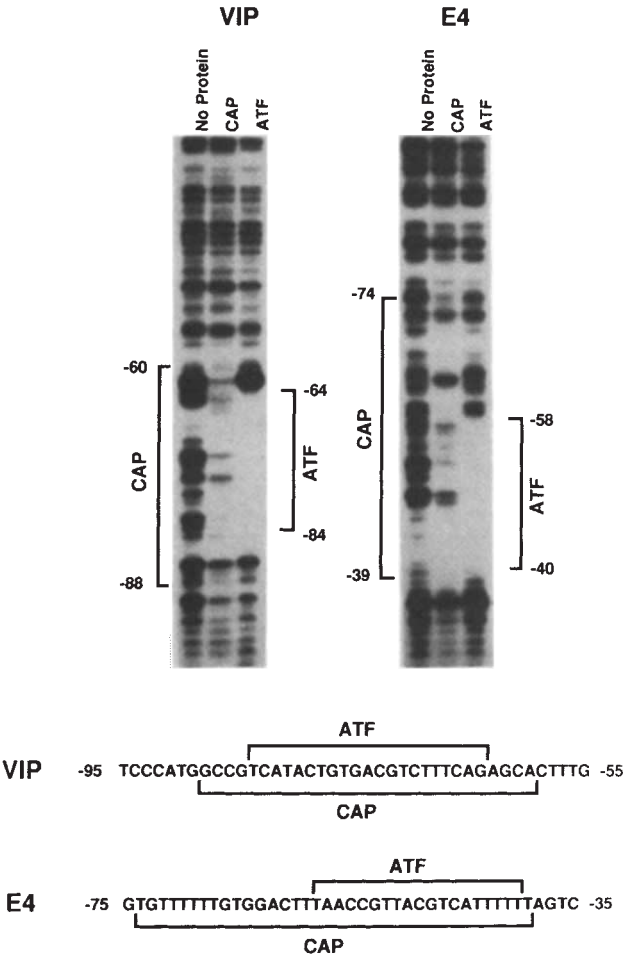


FIG. 3 Purified *E. coli* CAP binds to mammalian ATF sites. ³²P end-labelled DNA probes from the adenovirus E4 and VIP promoters were incubated with purified *E. coli* CAP protein or purified HeLa cell ATF followed by DNAase I digestion. The ³²P-end-labelled DNA probes used are indicated above the autoradiographs. The promoter regions protected from DNAase I digestion are indicated on the sides. The promoter sequence and regions of protection are shown below the autoradiographs.

METHODS. pE4BS and pVIPRN were previously described⁴. Methods as in the legend to Fig. 2, except that the probes used for footprinting were the *Hind*III-*Pvu*II fragments of pE4BS and pVIPRN 3' end-labelled at the *Hind*III site.

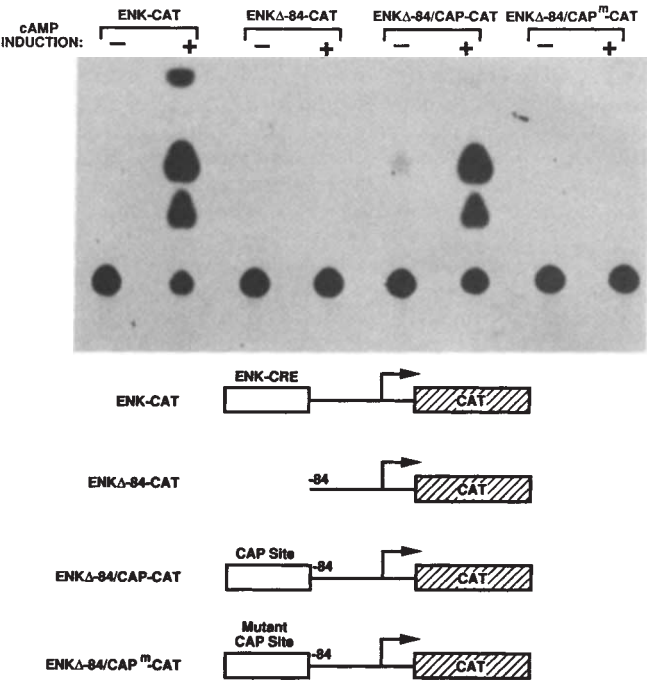


FIG. 4 An *E. coli* CAP site can confer cAMP-inducibility onto a heterologous gene in CV-1 cells. The ENK-CAT derivatives used are indicated above the autoradiograph and their structures are shown below. METHODS. pENK-CAT and pENKΔ-84-CAT (gift from M. Comb) were previously described¹⁴ and referred to as pENKAT-12 and pENKΔ-84, respectively. pENKΔ-84/CAP-CAT was constructed by inserting *E. coli* CRP gene sequences +25 to +62 between the *Sac*I and *Pst*I sites of pENKΔ-84-CAT. pENKΔ-84/CAP^m-CAT was constructed by inserting between the *Sac*I and *Pst*I sites a double-stranded synthetic oligonucleotide containing *E. coli* CRP gene sequences from +25 to +62, except that the CAP site at position +50 to +46 was mutated from 5'-TGTGA-3' to 5'-TGGAC-3'. Transient transfection assays were performed in CV-1 cells. The transfection, cAMP-induction and CAT assays were performed as described by Comb *et al.*¹⁴ except that cAMP-induction was performed with 25 μM Forskolin and 0.5 mM IMX.

responsive promoter element has been evolutionarily conserved. Consistent with this possibility is that ATF DNA binding activity is widely distributed throughout the eukaryotes⁷.

We have identified promoters containing overlapping consensus ATF and *E. coli* CAP sites in a wide variety of eukaryotes (Table 1). The cAMP-inducible human VIP promoter (-78 TGTGACGTCTTTCAGA -63) and the mouse Aα promoter (-131 TGTGACGTCTTTCAGA -116) contain CAP sites with

TABLE 1 CAP/ATF overlap in eukaryotic promoters			
Organism	Promoter	Sequence	Position
Human	VIP	TGTGACGTCT	-78/-69
	BSF2/IL-6	TGTGACGTCC	-218/-227
	apolipoprotein CII	TGTGACGTGA	-169/-160
Adenovirus	E1a	TGTGACGTGG	-437/-428
	E3	TGTGACGAAA	-53/-62
	E4	TGTGACGTGG	-268/-259
Mouse	Aα	TGTGACGTCA	-131/-122
	NGF	TGTGACGAGC	-72/-63
Chicken	U4	TGTGACGTAG	-57/-48
<i>S. cerevisiae</i>	PGK	TGTGACGAAA	-416/-425
	CDC4	TGTGACGTTT	-222/-231

The table shows the partial sequences of 11 eukaryotic promoters that contain overlapping consensus ATF and CAP binding sites identified in a random literature search. The CAP and ATF binding elements are shown by brackets. Primary refs for promoter sequences available on request.

dyad symmetry, which match the consensus better than any natural *E. coli* CAP site (reviewed in ref. 1).

The possibility that a regulatory promoter element has been evolutionarily conserved is not without precedent. Genetic and physical evidence indicate that phage 16-3 of the nitrogen-fixing bacterium *Rhizobium meliloti* is unrelated to the lamboid phage 434. Sequence comparisons and functional studies, however,

have shown that some operators of these two phages are similar²⁰. Another example is the eukaryotic heat-shock element, which is identical in all eukaryotes. Interestingly, the heat-shock transcription factors, which recognize heat-shock elements, are regulated differently in higher and lower eukaryotes. The heat-shock factors may be less conserved than the regulatory promoter elements²¹⁻²³. □

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Detergent structure in crystals of a bacterial photosynthetic reaction centre

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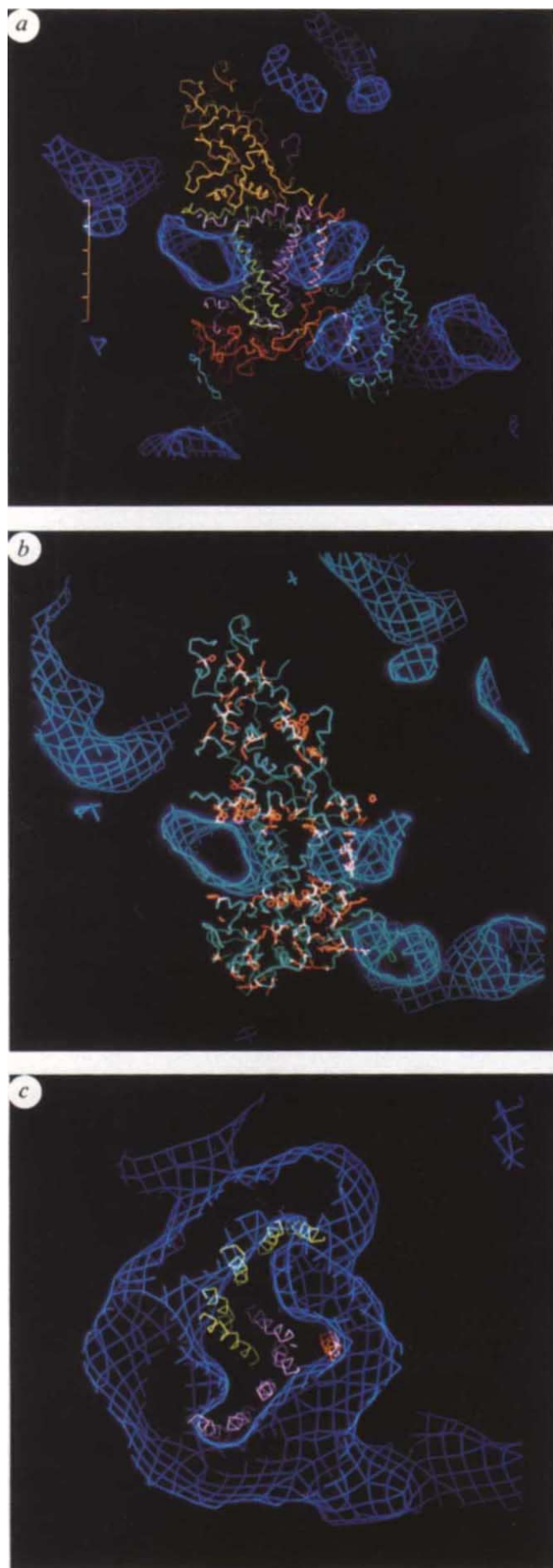
RECENT evidence shows that membrane-bound proteins can be crystallized successfully in the presence of detergent¹⁻³, which seems to facilitate the ordered packing of the proteins by binding to their hydrophobic surfaces in micellar manner^{4,5}. This approach has enabled the molecular structures of two bacterial photosynthetic reaction centres to be solved at high resolution by X-ray crystallography⁶⁻⁹, each of which has provided insights into the mechanism of photo-activated electron transport across the cell membrane. The detergent, however, although present in high concentration in the crystals, is not seen in these high-resolution structures because of disordering. To determine the structural motifs formed by the detergent that are involved in crystal packing, we have therefore generated a low-resolution structure using neutron diffraction with contrast variation. We find that the detergent is concentrated in rings which fill all the available space around the membrane-spanning α -helices of the reaction-centre protein subunits L, M and H. These rings are interconnected throughout the crystal lattice by short cylindrical detergent bridges such that zig-zag chains are formed parallel to the *c* direction. The average structure of the detergent therefore is spatially complementary to the structure of the reaction-centre complex and provides a model for the interaction between the lipid bilayer and the complex *in vivo*.

Because of disorder of the detergent in the reaction centre crystal (only one detergent molecule could be localized in the X-ray diffraction analysis) (J.D. *et al.*, in preparation), its structure could only be determined as a molecular average at low resolution. To analyse the structure we used low resolution

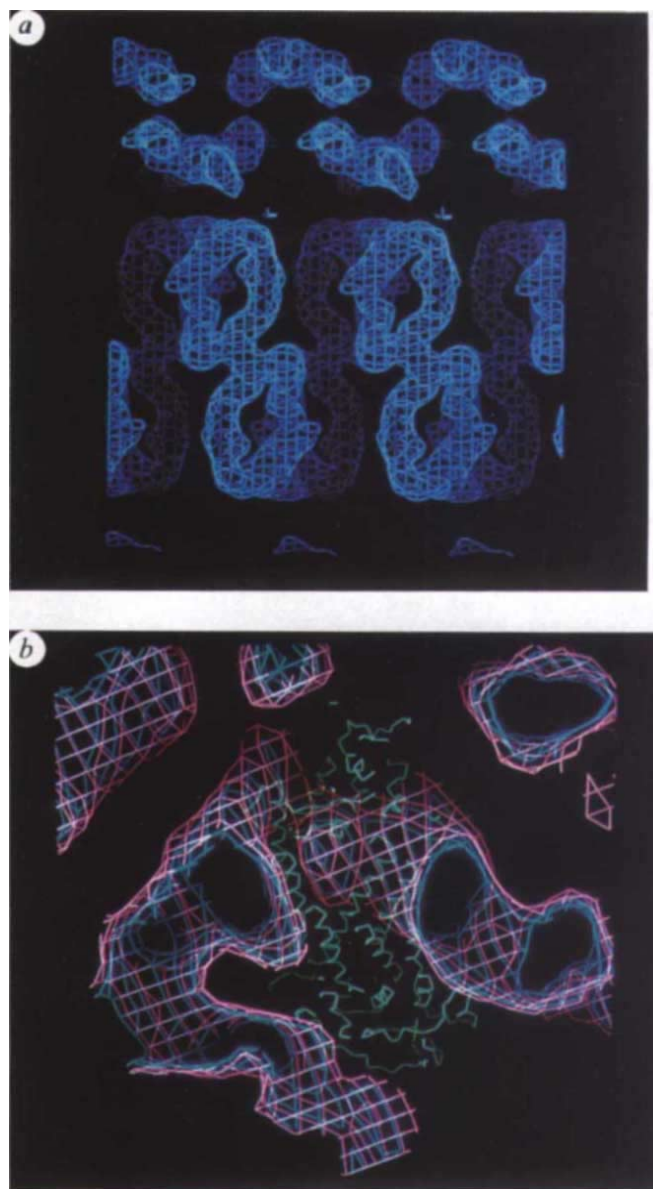
neutron diffraction with H₂O/D₂O contrast variation¹⁰. This consists of diffraction measurements from several reaction centre crystals, differing in their H₂O/D₂O content and thus in the relative contrast of the reaction centre molecule and the detergent with respect to the solvent.

The main quantitative information contained in contrast variation data is the variation of the phase of the structure factors as a function of contrast¹¹. The method is most useful in the case of a partially known structure. The phases of the complete structure at any contrast can be derived from the phases of the known part of the structure at a contrast where the scattering density of the unknown part is matched out by the solvent density. Details of the phasing method are described elsewhere¹². The result of the calculation is a statistical best estimate of the structure factors in any contrast, from which the density maps are then calculated. Results are summarized in Table 1.

The detergent is most clearly seen at a contrast close to 40% D₂O where the average protein density is matched out. At this contrast, the detergent has a relatively large negative contrast and its structure can be seen using an appropriate contouring level. The most striking feature we found is that the detergent appears as a distinct phase in the aqueous solvent region, forming a micelle around the protein, very much like the model proposed from small angle X-ray scattering data by Sardet *et al.*¹³ for the rhodopsin-*N,N* dimethyldodecylamine *N*-oxide (LDAO) complex. A reaction centre molecule from *Rhodospseudomonas viridis* consists of (1) a cytochrome *c* subunit at the periplasmic side of the membrane *in vivo* (at the top in Fig. 1); (2) a large H subunit at the cytoplasmic side *in vivo* (at the bottom in Fig. 1); and (3) in between, two subunits L and M, each forming five long, almost parallel, membrane-spanning α -helices⁷. These are connected by segments in contact with the cytochrome and the H subunit respectively, which form a surface parallel to the membrane plane and perpendicular to the helices (the limiting surfaces, Fig. 1*a* and *b*). The bulk of the detergent phase appears concentrated in rings around each reaction centre molecule at the level of the membrane-spanning α -helices of L, M, and H. The average plane of each ring is perpendicular to the approximate 2-fold axis relating the trans-membrane α -helices of subunits L and M. Another protein-detergent contact is found between the H subunit in the region H48-H55, which is poorly defined in the X-ray map, and the detergent ring of a neighbouring reaction centre molecule. An ordered LDAO molecule was found in the refined X-ray map (J.D. *et al.*, in preparation); its aliphatic chain lies well within the detergent density and its polar head outside.



◀ FIG. 1



▲ FIG. 2

FIG. 1 View of the reaction centre molecule surrounded by detergent (in blue); subunit H, orange; subunit M, mauve; subunit L, green; cytochrome, yellow; pale blue: parts of neighbouring molecules. The scale bar is marked every 10 Å. *a*, Only the C α backbone is shown. The transmembrane α -helices are around 40 Å long, but as none of them is perpendicular to the detergent ring, the average distance between the 'limiting surfaces' is 25–30 Å. The detergent ring cross-section is smaller in the region of the H transmembrane α -helix, where it is limited by hydrophilic regions of the neighbouring reaction centre molecule. *b*, The protein surface is rich in Trp, Arg and His residues, especially on the 'limiting surfaces', where we observe these side-chains in intimate contact with the detergent surface. The reaction centre C α backbone is in green, with the relevant side-chains in red. *c*, A cross-section through the reaction centre at the median level of the detergent ring showing the α -helices surrounded by the detergent; colours as in *a*.

FIG. 2 The packing of the reaction centre molecules in the P4₃2₁2 lattice can be described as a helical stacking of pairs of reaction centre molecules in two rows per unit cell parallel to [0, 0, 1]. One of these rows is centred at (0, 0, 0) and the other at (1/2, 1/2, 0), related by 4₃ symmetry. The detergent rings of the molecules within a single row are connected two by two through short cylindrical detergent contacts, or 'bridges', with a similar cross-section as the rings themselves. These bridges are located on crystallographic twofold axes and are of two types, a straight one and a hairpin one. The detergent is thus distributed in parallel zig-zag chains of interconnected rings throughout the crystal. These chains, parallel to the *c* axis, are only very loosely connected between them through weak detergent density found in the vicinity of the 4₃ crystal axes. *a*, View (down 110) of several unit cells showing the packing of the detergent rings within the crystal lattice, showing a zig-zag chain along the 2₁ axis parallel to *c*. *b*, View of one detergent ring, with the transmembrane helices of L, M and H subunits shown schematically in green, connected through one straight (at the right) and one hairpin (at the left) bridge to neighbouring rings. Two contour levels are shown: at σ (blue) and at 0.5 σ (mauve), where σ is the r.m.s. of the neutron scattering length density, demonstrating that the cross-section of the detergent ring is fairly insensitive to the choice of contouring level and that the bridges are probably not artefacts.

The detergent ring thickness perpendicular to the membrane plane is about 25–30 Å, which is close to the value given for LDAO micelles in pure water¹³ and is of the order of twice the length of an extended LDAO molecule (15–16 Å). This suggests that the LDAO molecules in the immediate neighbourhood of the protein are oriented parallel to the protein as in a lipid bilayer. It is remarkable that, all around the reaction centre molecule, the detergent ring fills the entire space available between the limiting surfaces of subunits L and M. This could indicate that (as much as the detergent-protein interaction is a valid model for membrane-protein interaction) in the native membrane the contact surface between the transmembrane helices and the membrane is only 25–30 Å wide, ending on either side at the limiting surfaces formed by the more hydrophilic parts of L and M. If the membrane is ≥ 40 Å thick on average¹⁴, it will have to rearrange to fit into the available space around the transmembrane helices of the reaction centre structure, in a more complicated way than the simple cylindrical model proposed by Yeates *et al.*¹⁵. In the native photosynthetic membrane the reaction centre is surrounded by light-harvesting complexes^{16,17}, whose interaction with the reaction centre protein may in fact be represented by some of the protein-detergent contacts in the present crystal structure.

The detergent rings are interconnected throughout the crystal as shown in Fig. 2. This network constitutes a three-dimensional ordered structure of a liquid-state phase, which is reminiscent of the structure of amphiphilic liquid crystalline phases. We have here a three-phase system where the three interfaces (detergent-protein, detergent-solvent, solvent-protein) are of similar extent and play an important role in the energy of the system. The detergent as defined by the contouring level shown in Fig. 1, occupies 13–14% of the unit cell volume. With a volume of 480 Å (ref. 3) per LDAO molecule, this would correspond to a concentration of the order of 0.5 M for the detergent in the

TABLE 1 Summary of data and statistical results of phasing and modelization

Data:				
Contrast measured (% D ₂ O)	0%	15%	55%	87%
R_{sym} (550 unique reflections)	0.036	0.078	0.037	0.055
Structure determination:				
Contrast used for phasing (% D ₂ O)	0%	40%		87%
Initial R -factors ($F_{\text{calc}}^{(1)}$ versus F_{obs})	0.19	0.64		0.64
Final R -factors (F_{best} versus F_{obs})	0.05	0.23		0.27
Average phase change (deg)	10.7	51.4		61.9
FOM after first F_{best} calculation	0.808	0.714		0.833
Refinement:				
Initial R -factors ($F_{\text{calc}}^{(2)}$ versus F_{obs})	0.19	0.36		0.46
FOM after iteration of F_{best} calculation	0.834	0.764		0.861

Crystals of the reaction centre from *Rps viridis* were grown as described earlier³ (space-group P4₃2₁2 with $a = b = 223.5$ Å and $c = 113.6$ Å) in 2.5 M ammonium sulphate. The crystals are parallelepipeds typically $0.4 \times 0.4 \times 0.8$ mm³ in size, the long dimension corresponding to the *c*-axis. The diffraction experiments were performed using crystals with four different contrasts, obtained by soaking them in a buffer of the same chemical composition as the mother liquor, but with a different D₂O/H₂O concentration (0, 15, 55, and 87% D₂O). The detergent used in these crystals was LDAO whose average scattering density is very close to that of H₂O containing 2.5 M ammonium sulphate. The concentration of zero contrast between solvent and detergent is thus very close to 0% D₂O, while at 40% D₂O the protein density is matched by the solvent. Neutron diffraction experiments were carried out on D17 and DB21, at the Institute Laue-Langevin (ILL), Grenoble (France). Both instruments use fairly long wavelength neutrons (11 Å and 7.5 Å respectively) and two-dimensional flat position sensitive detectors (BF₃ gas detector¹⁸ and Anger camera scintillation detector¹⁹ respectively). The data collection was made using stationary step scans with 0.15 deg between steps (typically 10 min per step). The data reduction was performed using a chain of programmes developed at ILL (refs 20, 21). R_{sym} is defined as $\Sigma (|I - \langle I \rangle|) / \Sigma I$, where I is the intensity of an individual reflection, $\langle I \rangle$ the average over symmetry equivalent reflections, the summation being over all reflections. Phasing: $F_{\text{calc}}^{(1)}$ corresponds to the known X-ray structure. As expected, the agreement is best at 0% D₂O contrast where the detergent is matched out. No adjustment or refinement of the protein model with respect to the measured data was performed, other than the calculation of resolution independent scale factors, one per data set. F_{best} is the modulus of the least-squares (best) estimator of the true F , resulting from the statistical combination of the F_{obs} and $F_{\text{calc}}^{(1)}$ probability distribution¹². The figure of merit (FOM) is the average of the cosine of the difference between the measured and calculated phase differences between the contrast under consideration and a reference contrast. $F_{\text{calc}}^{(2)}$ corresponds to the X-ray structure plus a detergent model deduced from the first 40% D₂O density maps. A marked improvement is seen for the contrasts where the detergent is not matched out, demonstrating the validity of this model. R -factors are calculated for reflections with $F_{\text{obs}} > 2\sigma(F_{\text{obs}})$.

crystal. With respect to molecular packing in the lattice, it is clear that the protein-protein interaction between the cytochromes and the H-subunits is important. The regions of protein-protein contact seem free of detergent density, although the detergent ring is touching one of the H-H contact regions. The detergent nevertheless plays a decisive role in the crystal packing. It prevents aggregation of the exposed apolar surfaces of the reaction center molecules in general and, more specifically, interacts tightly and stereo-specifically with the protein. This property may explain the pronounced dependence of membrane protein crystallization on the nature of the detergent used. □

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CORRECTIONS

The FC γ receptor of natural killer cells is a phospholipid-linked membrane protein

David Simmons & Brian Seed

Nature **333**, 568-570 (1988).

THIS letter reported the isolation and partial characterization of a cDNA clone encoding a phospholipid-anchored form of CD16/FcR γ III. We regret that the data purporting to show displacement of monomeric IgG1 cannot be reproduced, nor can the data showing phospholipid anchorage of the CD16 form found on peripheral-blood mononuclear cells that do not adhere to nylon wool. In addition recent studies have shown that there are at least two forms of CD16: a phospholipid-linked form expressed by granulocytes and a conventionally anchored form expressed by natural killer cells (L. Lanier *et al.* *J. Immun.* **141**, 3478-3485; 1988; B. J. Scallon *et al.* *Proc. natn. Acad. Sci. U.S.A.* **86**, 5079-5083; 1989; J. Ravetch & B. Perussia, personal communication).

The sequence of the cDNA clone, and its attachment to the cell surface by a phospholipid anchor, have been confirmed by further investigation. Jeffrey Ravetch, however, has kindly pointed out that the molecular weight marker scale in Fig. 2b gives inappropriately low values for the CD16 genomic DNA fragment lengths.

We would like to sincerely apologize for the confusion and misdirected effort these errors have provoked. □

Expression of a large family of POU-domain regulatory genes in mammalian brain development

Xi He, Maurice N. Treacy, Donna M. Simmons,
Holly A. Ingraham, Larry W. Swanson
& Michael G. Rosenfeld

Nature **340**, 35-42 (1989).

IN this Article there is an error in the amino acid sequence encoded by Tst-1 gene (hybridizing to 7.6, 4.4, 3.2 and 2.4 Kb brain mRNAs) reported in Fig. 1b, two residues before the end of POU-specific domain. The correct sequence is ...WLEETD... instead ...WLEEAD... □

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